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APPLICATION

FOR

UNITED STATES LETTERS PATENT

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APPLICANT : Bruce A. Littlefield, Monica H. Palme, Boris M. Seletsky, Murray J. Towle, Melvin J. Yu, and Wanjun Zheng

TITLE : Macrocyclic Analogs and Methods of Their Use and Preparation

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MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND PREPARATION

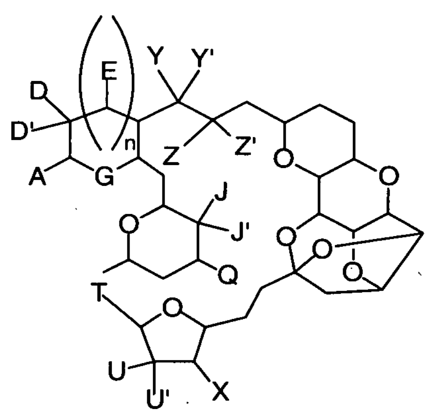
Background

The invention relates to pharmaceutically active macrolides. Halichondrin B is a potent anticancer agent originally isolated from the marine sponge *Halichondria okadai*, and subsequently found in *Axinella sp.*, *Phakellia carteri*, and *Lissondendryx sp.*.

A total synthesis of Halichondrin B was published in 1992 (Aicher, T.D. et al., *J. Am. Chem. Soc.* 114:3162-3164). Halichondrin B has demonstrated *in vitro* inhibition of tubulin polymerization, microtubule assembly, beta^{S}-tubulin crosslinking, GTP and vinblastine binding to tubulin, and tubulin-dependent GTP hydrolysis and has shown *in vitro* and *in vivo* anti-cancer properties.

Summary of the Invention

The invention provides halichondrin analogs having pharmaceutical activity, such as anticancer or antimitotic (mitosis-blocking) activity. These compounds are substantially smaller than halichondrin B. The invention features a compound having the formula (I):



Formula (I)

In formula (I), A is a C_{1-6} saturated or C_{2-6} unsaturated hydrocarbon skeleton, the skeleton being unsubstituted or having between 1 and 13 substituents, preferably between 1 and 10 substituents, e.g., at least one substituent selected from cyano, halo, azido, Q_1, and oxo. Each Q_1 is independently selected from OR_1, SR_1, SO_2R_1, OSO_2R_1, NR_2R_1,

$NR_2(CO)R_1$, $NR_2(CO)(CO)$[illegible]$NR_4(CO)NR_2R_1$, $NR_2(CO)OR_1$, (CO)[illegible], $O(CO)R_1$, $(CO)NR_2R_1$, and $O(CO)NR_2R_1$. The number of substituents can be, for example, between 1 and 6, 1 and 8, 2 and 5, or 1 and 4. Throughout the disclosure, numerical ranges are understood to be inclusive.

Each of R_1, R_2, R_4, R_5, and R_6 is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} aminoalkyl, C_{6-10} aryl, C_{6-10} haloaryl (e.g., p-fluorophenyl or p-chlorophenyl), C_{6-10} hydroxyaryl, C_{1-4} alkoxy-C_6 aryl (e.g., p-methoxyphenyl, 3,4,5-trimethoxyphenyl, p-ethoxyphenyl, or 3,5-diethoxyphenyl), C_{6-10} aryl-C_{1-6} alkyl (e.g., benzyl or phenethyl), C_{1-6} alkyl-C_{6-10} aryl, C_{6-10} haloaryl-C_{1-6} alkyl, C_{1-6} alkyl-C_{6-10} haloaryl, (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heterocyclic radical-C_{1-6} alkyl, C_{2-9} hydroxyheterocyclic radical, C_{2-9} heterocyclic radical-C_{1-3} alkylhydroxy, C_{2-9} heteroaryl, and C_{2-9} heteroaryl-C_{1-6} alkyl. There may be more than one R_1, for example, if A is substituted with two different alkoxy (OR_1) groups such as butoxy and 2-aminoethyoxy.

Examples of A include 2,3-dihydroxypropyl, 2-hydroxyethyl, 3-hydroxy-4-perfluorobutyl, 2,4,5-trihydroxypentyl, 3-amino-2-hydroxypropyl, 1,2-dihydroxyethyl, 2,3-dihyroxy-4-perflurobutyl, 3-cyano-2-hydroxypropyl, 2-amino-1-hydroxy ethyl, 3-azido-2-hydroxypropyl, 3,3-difluoro-2,4-dihydroxybutyl, 2,4-dihydroxybutyl, 2-hydroxy-2(p-fluorophenyl)-ethyl, $-CH_2(CO)$(substituted or unsubstituted aryl), $-CH_2(CO)$(alkyl or substituted alkyl, such as haloalkyl or hydroxyalkyl) and 3,3-difluoro-2-hydroxypent-4-enyl.

Examples of Q_1 include -NH(CO)(CO)-(heterocyclic radical or heteroaryl), $-OSO_2$-(aryl or substituted aryl), -O(CO)NH-(aryl or substituted aryl), aminoalkyl, hydroxyalkyl, -NH(CO)(CO)-(aryl or substituted aryl), -NH(CO)(alkyl)(heteroaryl or heterocyclic radical), O(substituted or unsubstituted alkyl)(substituted or unsubstituted aryl), and –NH(CO)(alkyl)(aryl or substituted aryl).

Each of D and D' is independently selected from R_3 and OR_3, wherein R_3 is H, C_{1-3} alkyl, or C_{1-3} haloalkyl. Examples of D and D' are methoxy, methyl, ethoxy, and ethyl. In some embodiments, one of D and D' is H.

The value for n is 1 or preferably 0, thereby forming either a six-membered or five-membered ring. This ring can be unsubstituted or substituted, e.g., where E is R_5 or OR_5, and can be a heterocyclic radical or a cycloalkyl, e.g. where G is S, CH_2, NR_6, or preferably O.

Each of J and J' is independently H, C_{1-6} alkoxy, or C_{1-6} alkyl; or J and J' taken together are $=CH_2$ or –O-(straight or branched C_{1-5} alkylene or alkylidene)-O-, such as exocyclic methylidene, isopropylidene, methylene, or ethylene. Q is C_{1-3} alkyl, and is preferably methyl. T is ethylene or ethenylene, optionally substituted with $(CO)OR_7$, where R_7 is H or C_{1-6} alkyl. Each of U and U' is independently H, C_{1-6} alkoxy, or C_{1-6} alkyl; or U and U' taken together are $=CH_2$ or –O-(straight or branched C_{1-5} alkylene or alkylidene)-O-. X is H or C_{1-6} alkoxy. Each of Y and Y' is independently H or C_{1-6} alkoxy; or Y and Y' taken together are =O, $=CH_2$, or –O-(straight or branched C_{1-5} alkylene or alkylidene)-O-. Each of Z and Z' is independently H or C_{1-6} alkoxy; or Z and Z' taken together are =O, $=CH_2$, or –O-(straight or branched C_{1-5} alkylene or

alkylidene)-O-.

The invention features compounds of sufficient stability to be suitable for pharmaceutical development. The invention also features pharmaceutically acceptable salts of disclosed compounds, disclosed novel synthetic intermediates, pharmaceutical compositions containing one or more disclosed compounds, methods of making the disclosed compounds or intermediates, and methods of using the disclosed compounds or compositions. Methods of use include methods for reversibly or irreversibly inhibiting mitosis in a cell, and for inhibiting cancer or tumor growth *in vitro*, *in vivo*, or in a patient. The invention also features methods for identifying an anti-mitotic or anti-cancer agent, such as a reversible or, preferably, an irreversible agent.

Brief Description of the Figures

FIG. 1 is a graph showing the percentage of cells which have completed mitosis and returned to the G_1 phase as a function of concentration of compound B1939 in a mitotic block assay. The minimum concentration required for complete mitotic block at 0 hour is 10 nM. The minimum concentration required for complete mitotic block at 10 hour (after washout) is also 10 nM. The reversibility ratio is therefore 1 for B1939. Superimposed upon this graph is a curve showing the percentage of viable cells at 5 days as a function of concentration of compound B1939. Viability drops to very low levels at the same concentration as the 10 hour mitotic block.

FIG. 2 is a graph showing the percentage of cells which have completed mitosis and returned to G_1 phase as a function of the concentration of compound B2042 in a mitotic block reversibility assay. The minimum concentration required for complete mitotic block at 0 hour is 3 nM. The minimum concentration required for complete mitotic block at 10 hour is 100 nM. The reversibility ratio for B2042 is therefore 33. Superimposed on this graph is a curve showing the percentage of viable cells at 5 days as a function of concentration of compound B2042. Viability drops to very low levels at the same concentration as the 10 hour mitotic block.

FIG. 3 is a graph showing the average tumor volume in microliters as a function of time (days) in a LOX melanoma xenograft growth inhibition assay. This illustrates the antitumor activity of a compound of formula (I), compound B1939. Paclitaxel and a vehicle control were used.

FIG. 4 is a graph showing the average body weight per mouse as a function of time (days) in the assay described in FIG. 3.

FIG. 5 is a graph showing the average tumor volume in microliters as a function of time (days) in a COLO 205 human colon cancer xenograft growth inhibition assay, showing the antitumor activities of vinblastine and vincristine.

A. Definitions
B. Halichondrin Analogs
C. Synthesis of Halichondrin Analogs
D. Pharmacological Activity
E. Uses

A. Definitions

The following terms are defined in part below and by their usage herein.

Hydrocarbon skeletons contain carbon and hydrogen atoms and may be linear, branched, or cyclic. Unsaturated hydrocarbons include one, two, three or more C-C double bonds (sp^2) or C-C triple bonds (sp). Examples of unsaturated hydrocarbon radicals include ethynyl, 2-propynyl, 1-propenyl, 2-butenyl, 1,3-butadienyl, 2-pentenyl, vinyl (ethenyl), allyl, and isopropenyl. Examples of bivalent unsaturated hydrocarbon radicals include alkenylenes and alkylidenes such as methylidyne, ethylidene, ethylidyne, vinylidene, and isopropylidene. In general, compounds of the invention have hydrocarbon skeletons ("A" in formula (I)) that are substituted, e.g., with hydroxy, amino, cyano, azido, heteroaryl, aryl, and other moieties described herein. Hydrocarbon skeletons may have two geminal hydrogen atoms replaced with oxo, a bivalent carbonyl oxygen atom (=O), or a ring-forming substituent, such as -O-(straight or branched alkylene or alkylidene)-O- to form an acetal or ketal.

C_{1-6} alkyl includes linear, branched, and cyclic hydrocarbons, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, sec-pentyl, neo-pentyl, tert-pentyl, cyclopentyl, hexyl, isohexyl, sec-hexyl, cyclohexyl, 2-methylpentyl, tert-hexyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1,3-dimethylbutyl, and 2,3-dimethyl but-2-yl. Alkoxy (-OR), alkylthio (-SR), and other alkyl-derived moieties (substituted, unsaturated, or bivalent) are analogous to alkyl groups (R). Alkyl groups, and alkyl-derived groups such as the representative alkoxy, haloalkyl, hydroxyalkyl, alkenyl, alkylidene, and alkylene groups, can be C_{2-6}, C_{3-6}, C_{1-3}, or C_{2-4}.

Alkyls substituted with halo, hydroxy, amino, cyano, azido, and so on can have 1, 2, 3, 4, 5 or more substituents, which are independently selected (may or may not be the same) and may or may not be on the same carbon atom. For example, haloalkyls are alkyl groups with at least one substituent selected from fluoro, chloro, bromo, and iodo. Haloalkyls may have two or more halo substituents which may or may not be the same halogen and may or may not be on the same carbon atom. Examples include chloromethyl, periodomethyl, 3,3-dichloropropyl, 1,3-difluorobutyl, and 1-bromo-2-chloropropyl.

Heterocyclic radicals and heteroaryls include furyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathienyl, 2H-pyrrolyl, pyrrolyl, imidazolyl (e.g., 1-, 2- or 4-imidazolyl), pyrazolyl, isothiazolyl, isoxazolyl, pyridyl (e.g., 1-, 2-, or 3- pyridyl), pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, indolyl (e.g., 1-, 2-, or 3-

indolyl), indazolyl, purinyl, [illegible]-quinolixinyl, isoquinolyl, quinolyl, p[illegible]alazinyl, naphthyridinyl, quinoxalinyl, quinazolyinyl, cinnolinyl, pteridinyl, pyrrolinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, indolinyl, isoindolinyl, and morpholinyl. Heterocyclic radicals and heteroaryls may be linked to the rest of the molecule at any position along the ring. Heterocyclic radicals and heteroaryls can be C_{2-9}, or smaller, such as C_{3-6}, C_{2-5}, or C_{3-7}.

Aryl groups include phenyl, benzyl, naphthyl, tolyl, mesityl, xylyl, and cumenyl.

It is understood that "heterocyclic radical", "aryl", and "heteroaryl" include those having 1, 2, 3, 4, or more substituents independently selected from lower alkyl, lower alkoxy, amino, halo, cyano, nitro, azido, and hydroxyl. Heterocyclic radicals, heteroaryls, and aryls may also be bivalent substituents of hydrocarbon skeleton "A" in formula (I).

B. Halichondrin Analogs

Referring to formula (I) in the Summary section, embodiments of the invention include compounds wherein n is 0; wherein each of D and D' is independently selected from R_3, C_{1-3} alkoxy, and C_{1-3} haloalkyloxy; wherein R_5 is selected from H, C_{2-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} aminoalkyl, C_{6-10} aryl, C_{6-10} haloaryl, C_{6-10} hydroxyaryl, C_{1-3} alkoxy-C_6 aryl, C_{6-10} aryl-C_{1-6} alkyl, C_{1-6} alkyl-C_{6-10} aryl, C_{6-10} haloaryl-C_{1-6} alkyl, C_{1-6} alkyl-C_{6-10} haloaryl, (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heterocyclic radical-C_{1-6} alkyl, C_{2-9} heteroaryl, and C_{2-9} heteroaryl-C_{1-6} alkyl; and combinations thereof.

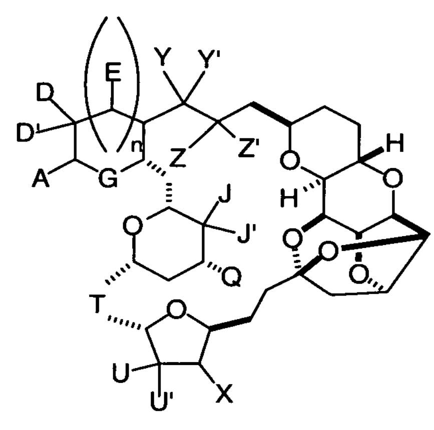
Other embodiment includes compounds having one or more of the following characteristics: (a) wherein A is a C_{1-6} saturated or C_{2-6} unsaturated hydrocarbon skeleton, the skeleton having at least one substituent selected from cyano, halo, azido, Q_1, and oxo; (b) each Q_1 is independently selected from OR_1, SR_1, SO_2R_1, OSO_2R_1, NR_2R_1, $NR_2(CO)R_1$, $NR_2(CO)R_1$, and $O(CO)NR_2R_1$; (c) Z and Z' taken together are =O or =CH_2; (d) wherein each Q_1 is independently selected from OR_1, SR_1, SO_2R_1, OSO_2R_1, $NH(CO)R_1$, $NH(CO)(CO)R_1$, and $O(CO)NHR_1$; (e) each R_1 is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_6 aryl, C_6 haloaryl, C_{1-3} alkoxy-C_6 aryl, C_6 aryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 aryl, C_6 haloaryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 haloaryl, (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heteroaryl, and C_{2-9} heteroaryl-C_{1-6} alkyl; (f) one of D and D' is methyl or methoxy and the other is H; (g) n is 0; (h) G is O; (i) J and J' taken together are =CH_2; (j) Q is methyl; (k) T is ethylene; (l) U and U' taken together are =CH_2 ; (m) X is H; (n) each of Y and Y' is H; and (o) Z and Z' taken together are =O. Examples of combinations are the combination of (h)-(m), the combination of (a) and (b), the combination of (f) and (h), and the combination of (h) and where one of D and D' is methyl and the other is H. Two particularly preferred compounds are B1793 and B1939.

Another embodiment includes compounds wherein Q_1 is independently selected from OR_1, SR_1, SO_2R_1, and OSO_2R_1; and each R_1 is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_6 aryl, C_6 haloaryl, C_{1-3} alkoxy-C_6 aryl, C_6 aryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 aryl, C_6 haloaryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 haloaryl, and (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl. Other

embodiments include compounds wherein: one of D and D' is alkyl or alkoxy, where n is 1; (f) as above, where n is 1; E is alkoxy, where n is 1; n is 0, where one of D and D' is hydroxy and the other is H; and (f) as above, where n is 1 and E is methyl.

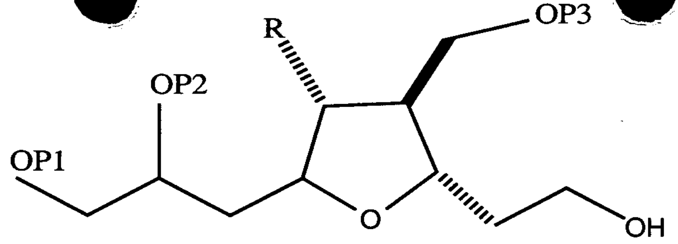
The invention also features compounds wherein: (1) A has at least one substituent selected from hydroxyl, amino, azido, halo, and oxo; (2) A is a saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl, amino, and azido (e.g., B1793, B1939, B2042, B1794, and B1922); (3) A has at least two substituents independently selected from hydroxyl, amino, and azido (e.g., B2090 and B2136); (4) A has at least two substituents independently selected from hydroxyl and amino (e.g., B2042 and B2090); (5) A has at least one hydroxyl substituent and at least one amino substituent (e.g., B1939 and B2136); (6) A has at least two hydroxyl substituents (e.g., B1793 and B1794); (7) A is a C_{2-4} hydrocarbon skeleton that is substituted (e.g., B2004, B2037, B1920, B2039, B2070, B2090, and B2043); (8) A is a C_3 hydrocarbon skeleton that is substituted (e.g., B1793, B1920, B1984, B1988, B1939, B1940, B2014); (9) A has an (S)-hydroxyl alpha to the carbon atom linking A to the ring containing G (e.g., B1793, B1939 or B1920) or an (R)-hydroxyl (e.g. B2102, B2013, B2042); and (10) A is a C_{1-6} saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl and cyano (e.g., B2013, B2037, B2102, B2086, and B2091). By (S)-hydroxyl is meant the configuration of the carbon atom having the hydroxyl group is (S). Embodiments of the invention also include compounds which have at least two substituents on the carbon atoms (1) alpha and gamma, (2) beta and gamma, or preferably (3) alpha and beta to the carbon atom linking A to the ring containing G. The alpha, beta, and gamma carbon atoms can have an (R) or (S) configuration

The invention further provides preferred compounds having the formula (1) -A, shown below, wherein the substituents are identical to those defined above.



Formula 1-A

The invention further features the following monosaccharide intermediate having formula (II):



Formula (II)

wherein R is methyl or methoxy, and each of P1, P2, and P3 is independently selected from H and primary alcohol protecting groups. Preferably, the diol sidechain is below the plane of the page and OP2 is above the plane of the page. Primary alcohol protecting groups include esters, ethers, silyl ethers, alkyl ethers, and alkoxyalkyl ethers.

Examples of esters include formates, acetates, carbonates, and sulfonates. Specific examples include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, pivaloate, crotonate, 4-methoxy- crotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate, carbonates such as methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl)ethyl, vinyl, allyl, and p-nitrobenzyl.

Examples of silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkylsilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, allyl, and allyloxycarbonyl ethers or derivatives. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrahydropyranyl ethers. Examples of benzyl ethers include p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-picolyl. Preferably, each of P1 and P2 are TBS and P3 is MPM (see alcohol **19** below). In one aspect, formula (II) can be modified so the hydroxyethyl sidechain can also be a protected hydroxyl, $-CH_2CH_2O$-P4, wherein P4 is independently selected from values for P1. A related intermediate is alcohol **17**, where the hydroxyethyl sidechain is a hydroxymethyl sidechain. A corresponding hydroxypropyl sidechain, or aminoalkyl side chain, can be similarly prepared.

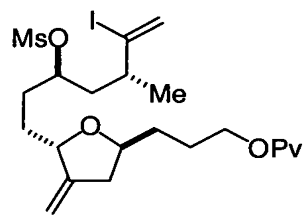
P1 and P2, taken together, can be a diol protecting group, such as cyclic acetals and ketals (methylene, ethylidene, benzylidenes, isopropylidene, cyclohexylidene, and cyclopentylidene), silylene derivatives such as di-t-butylsilylene and 1,1,3,3-tetra-isopropylidisiloxanylidene derivatives, cyclic carbonates, and cyclic boronates. Methods of adding and removing such hydroxyl protecting groups, and additional protecting groups, are well-known in the art and available, for example, in P.J. Kocienski, Protecting Groups, Thieme, 1994, and in T.W. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 2^{nd} edition, John Wiley & Sons, 1992.

The following section provides representative syntheses of intermediates of formula (II) and halichondrin analogs of formula (I).

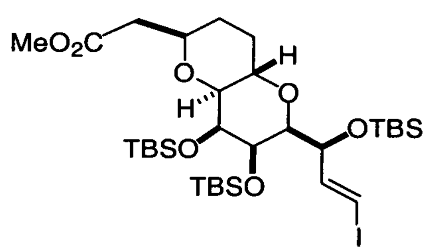
C. Synthesis of Halichondrin Analogs

An overview is provided below, followed by synthetic schemes 1-16, and several detailed protocols.

Compounds of general formula **4** can be prepared by the route outlined in Scheme 1. Key fragment **F-2** exemplified by vinyl iodide compound **X2** can be prepared according to the procedure of Kishi, et al (Total synthesis of halichondrin B and norhalichondrin B. Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, . M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* **1992**, *114*, 3162-4).



Vinyl Iodide X2



XF3

Key fragment **F-3** can be obtained by DIBALH reduction of the corresponding methyl ester, XF3, prepared according to the procedure of Stamos, et al (Scheme 2). [Synthetic studies on halichondrins: a practical synthesis of the C.1-C.13 segment. Stamos, D. P.; Kishi, Y. *Tetrahedron Lett.* **1996**, *37*, 8643-8646]. Synthesis of key fragment **F-1** exemplified by compound **20** can be synthesized as described in Scheme 3 or Scheme 4.

Using **B1793** as a representative example, coupling of the three key fragments proceeded as outlined in Scheme 5: Nozaki-Hiyama-Kishi coupling of fragments 20 and X2 followed by intramolecular Williamson ether formation furnished tetrahydropyran **B2318**. Protecting group modification as described in Scheme 5 or alternatively in Scheme 6 afforded primary iodide **B2313**. Halogen-metal exchange reaction and coupling with key fragment **F-3** furnished a mixture of diastereomeric alcohols **B2308**. Additional protecting group manipulation and oxidation followed by an intramolecular Nozaki-Hiyama-Kishi reaction

afforded an intermediate, which when oxidized and treated with TBAF underwent intramolecular hetero-Michael ring closure. PPTs mediated acetal formation furnished **B1793**.

Aryl groups can be incorporated into the C32 sidechain (e.g. **B2043**) as exemplified in Scheme 7. Intermediate **B2318** was deprotected and the resulting diol oxidatively cleaved to the corresponding aldehyde. Treatment with a Grignard reagent (e.g. p-F-PhMgBr), separation of the resulting diastereomers and silylation furnished **204**, which was converted to final product in a manner similar to that described in Scheme 6.

Ether analogs can be prepared from **B1793** by treatment with an appropriate alkylating agent (e.g. Scheme 8). Similarly, sulfonates, esters, carbamates, etc. can be prepared from **B1793** by treatment with an activated carbonyl component. Oxidative diol cleavage and reduction or selective hydroxyl group oxidation could furnish derivatives such as **B2037** and **B1934**, respectively.

Alternatively, one or more hydroxyl groups could be converted to the corresponding amino groups with subsequent coupling with an activated carbonyl component (Scheme 9). Displacement of the sulfonyl intermediate (e.g. **B1920**) by carbon or heteroatom nucleophiles could also be readily accomplished (Scheme 10).

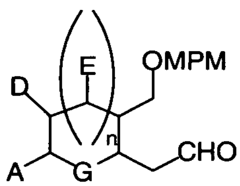
C31 methyl analogs can be prepared as outlined in Scheme 11. Indium mediated coupling of an allyl bromide ester with 2,3-*O*-(3-isopropylidine)-D-glyceraldehyde furnished lactone **103**. Hetero Michael addition, lactone reduction, Wittig coupling and intramolecular Michael addition furnished tetrahydrofuran **107**. Pummerer rearrangement, protecting group adjustment and DIBALH reduction furnished key fragment **1** (e.g., **114**), which was converted to final compound in a manner analogous to that described in Scheme 6.

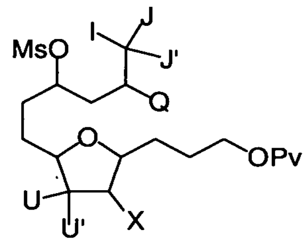
Fluorine atoms could be introduced as described in Schemes 12 - 14. Beginning with the appropriate tetrahydrofuran intermediate, fluorinated key fragment **1** was obtained and carried to final compound in a manner analogous to that illustrated in Scheme 6.

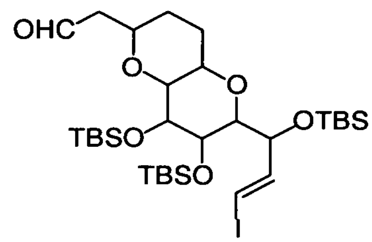
Triol derivatives could be similarly prepared from the tetrahydrofuran intermediate. For example, as outlined in Scheme 15 allyltributylstannane addition to aldehyde **X32** furnished homoallylic alcohol **33** that was carried to final compound in a similar manner to that described in Scheme 6. These triols could be further modified as exemplified in Scheme 6.

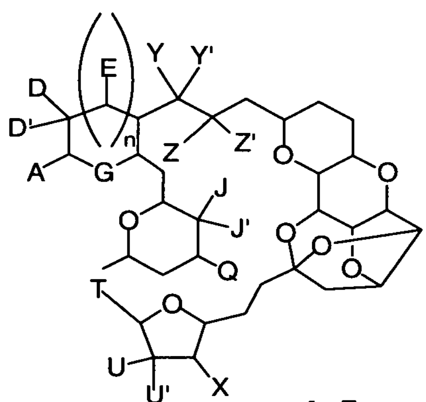
The 1,3 diol derivatives could be prepared from intermediates previously described. For example, **B2086** could be oxidatively cleaved and reduced to afford 1,3-diol **B2091** (Scheme 16).

Scheme 1

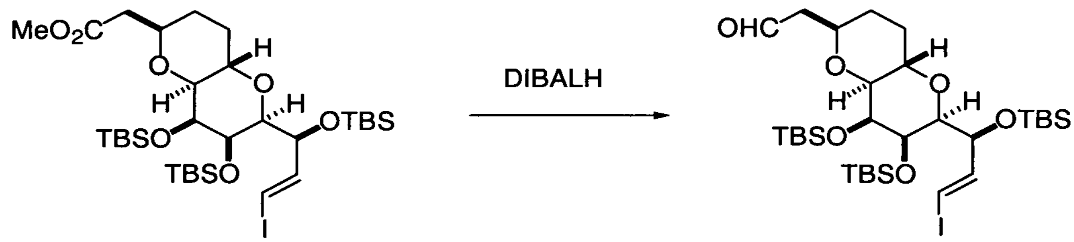

F-1


F-2


F-3

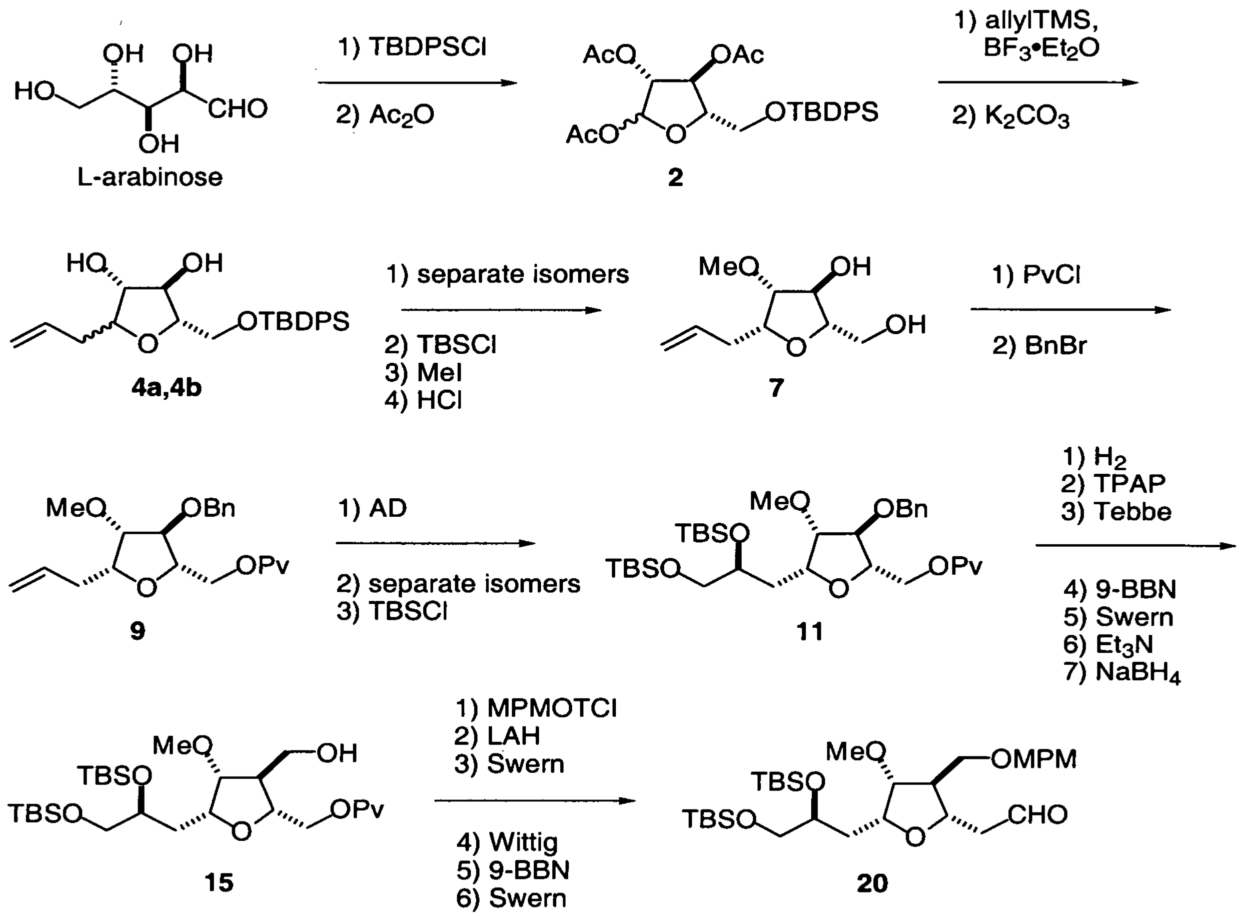

4 , Formula (I)

Scheme 2



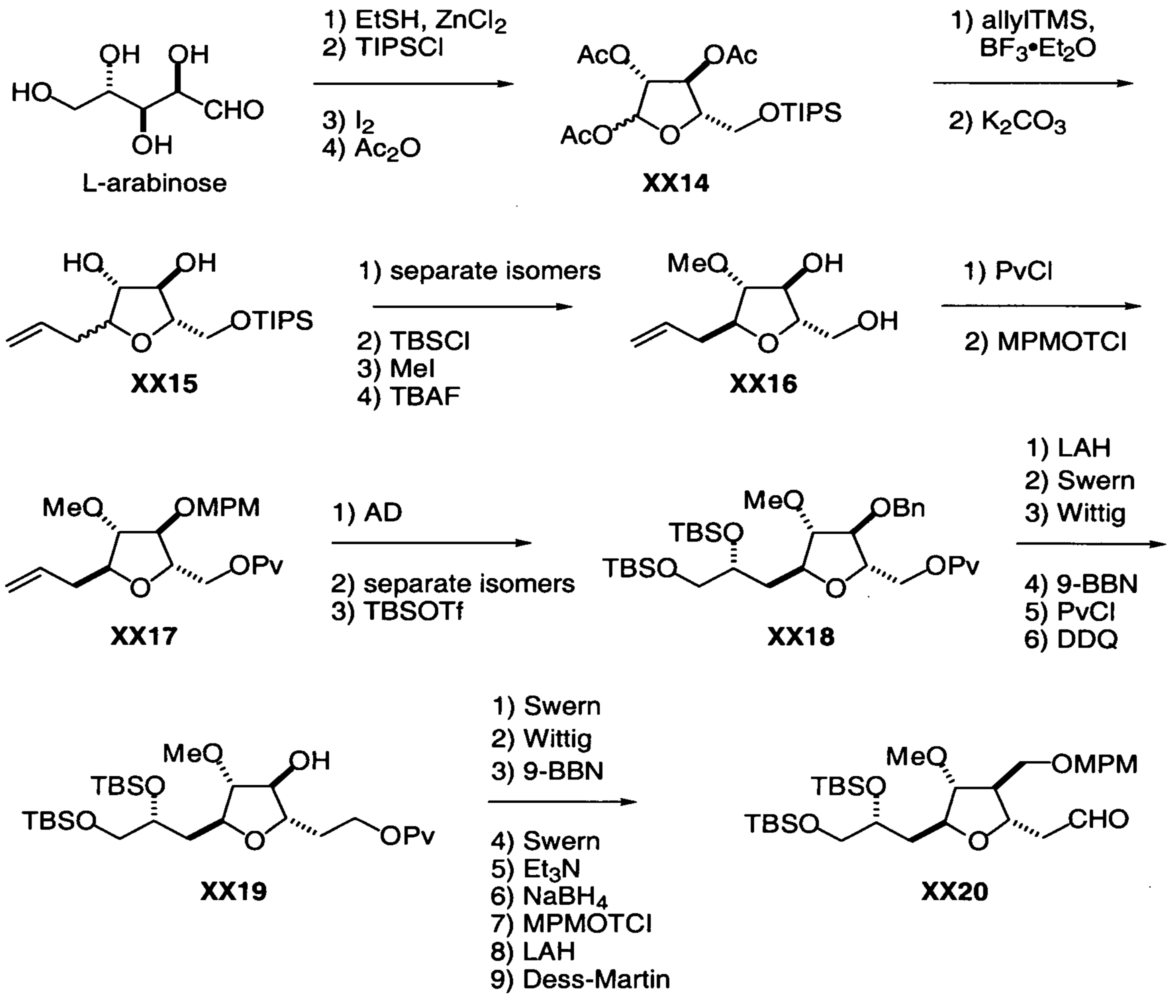
XF-3 **F-3**

Scheme 3

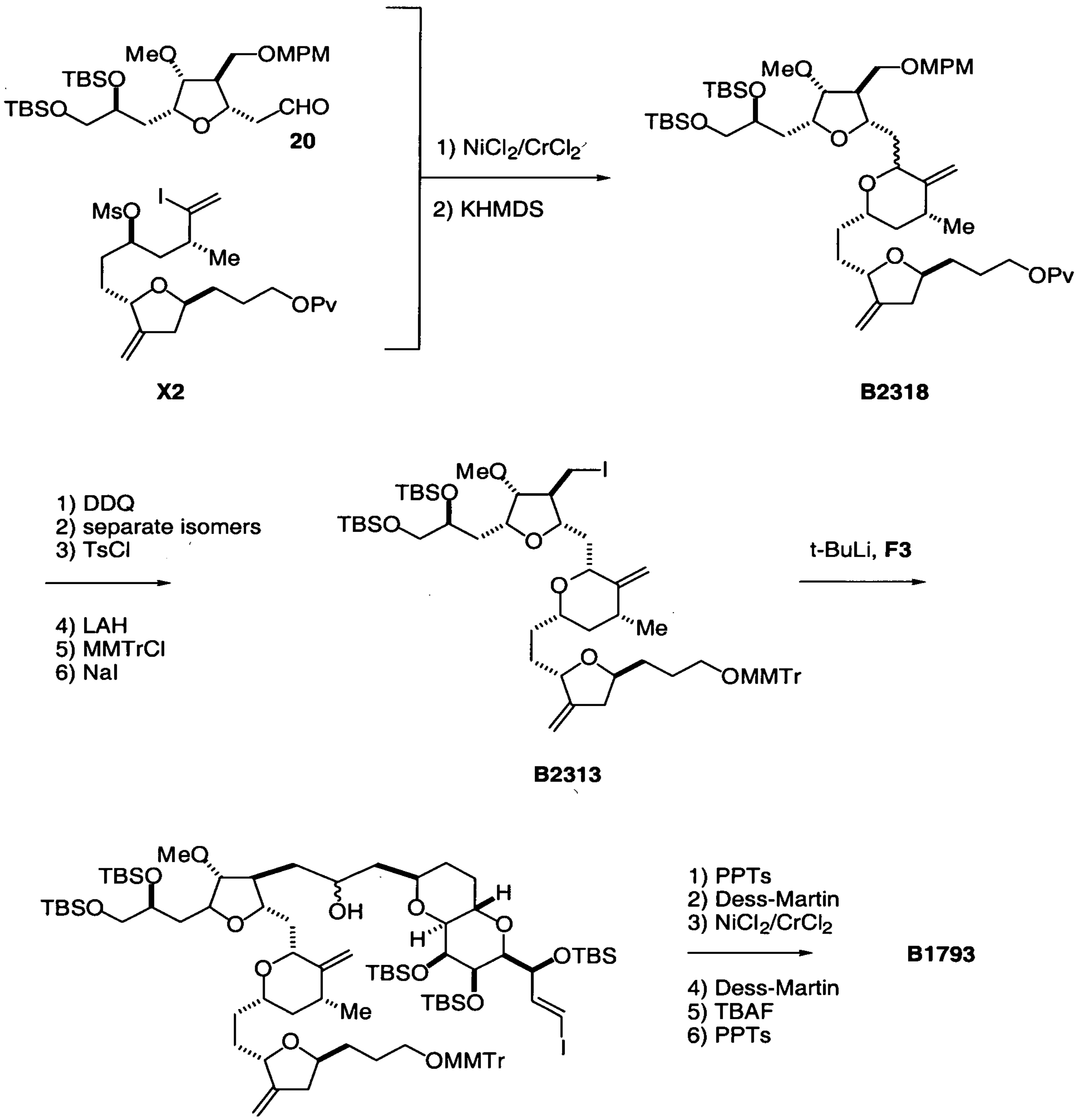


L-arabinose
1) TBDPSCl
2) Ac_2O
2
1) allylTMS, BF_3•Et_2O
2) K_2CO_3
4a,4b
1) separate isomers
2) TBSCl
3) MeI
4) HCl
7
1) PvCl
2) BnBr
9
1) AD
2) separate isomers
3) TBSCl
11
1) H_2
2) TPAP
3) Tebbe
4) 9-BBN
5) Swern
6) Et_3N
7) $NaBH_4$
15
1) MPMOTCl
2) LAH
3) Swern
4) Wittig
5) 9-BBN
6) Swern
20

Scheme 4

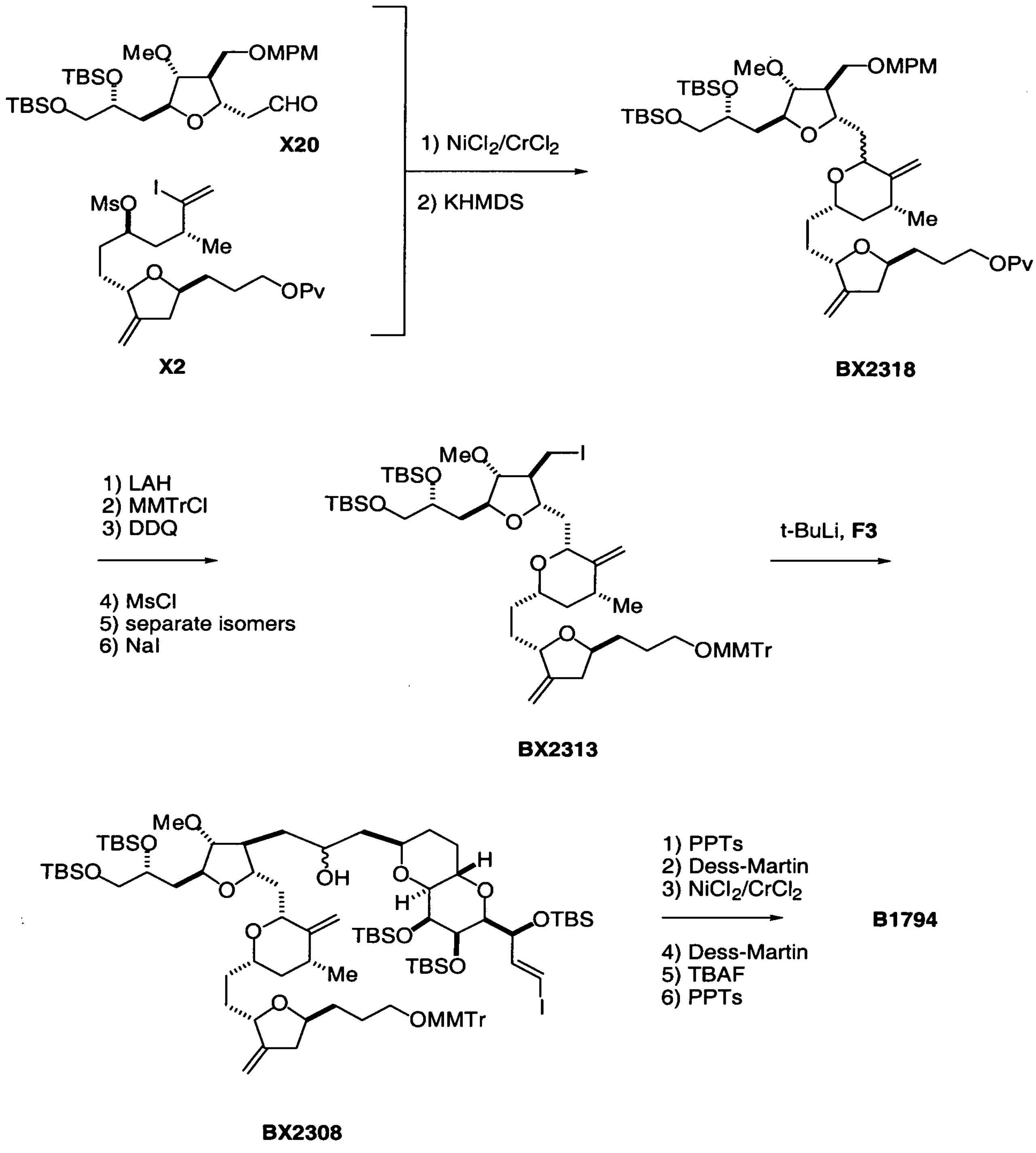

OH
OH
HO
CHO
OH
L-arabinose
1) EtSH, $ZnCl_2$
2) TIPSCl
3) I_2
4) Ac_2O
AcO
OAc
OTIPS
AcO
O
XX14
1) allylTMS, BF_3•Et_2O
2) K_2CO_3
HO
OH
OTIPS
O
XX15
1) separate isomers
2) TBSCl
3) MeI
4) TBAF
MeO
OH
OH
O
XX16
1) PvCl
2) MPMOTCl
MeO
OMPM
OPv
O
XX17
1) AD
2) separate isomers
3) TBSOTf
MeO
OBn
TBSO
TBSO
OPv
O
XX18
1) LAH
2) Swern
3) Wittig
4) 9-BBN
5) PvCl
6) DDQ
MeO
OH
TBSO
TBSO
OPv
O
XX19
1) Swern
2) Wittig
3) 9-BBN
4) Swern
5) Et_3N
6) $NaBH_4$
7) MPMOTCl
8) LAH
9) Dess-Martin
MeO
OMPM
TBSO
TBSO
CHO
O
XX20

Scheme 5

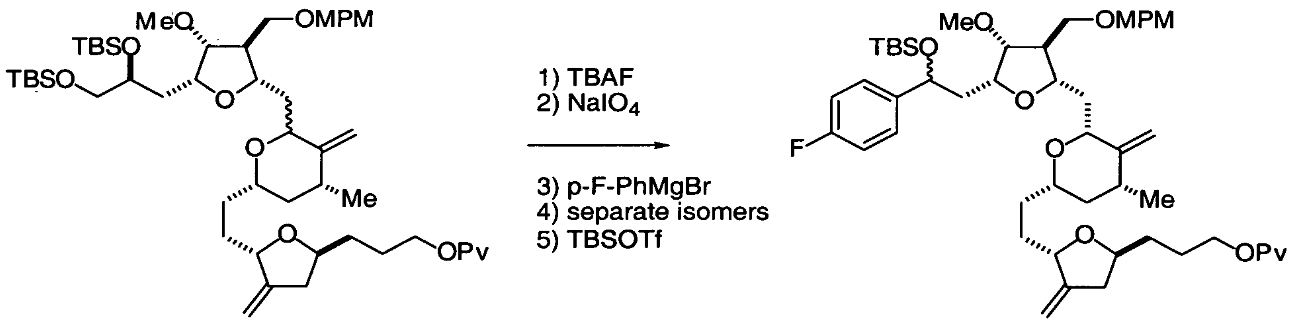


20
X2
1) NiCl2/CrCl2
2) KHMDS
B2318
1) DDQ
2) separate isomers
3) TsCl
4) LAH
5) MMTrCl
6) NaI
B2313
t-BuLi, F3
B2307, B2308
1) PPTs
2) Dess-Martin
3) NiCl2/CrCl2
4) Dess-Martin
5) TBAF
6) PPTs
B1793

Scheme 6


X20
X2
1) NiCl2/CrCl2
2) KHMDS
BX2318
1) LAH
2) MMTrCl
3) DDQ
4) MsCl
5) separate isomers
6) NaI
BX2313
t-BuLi, F3
BX2308
1) PPTs
2) Dess-Martin
3) NiCl2/CrCl2
4) Dess-Martin
5) TBAF
6) PPTs
B1794

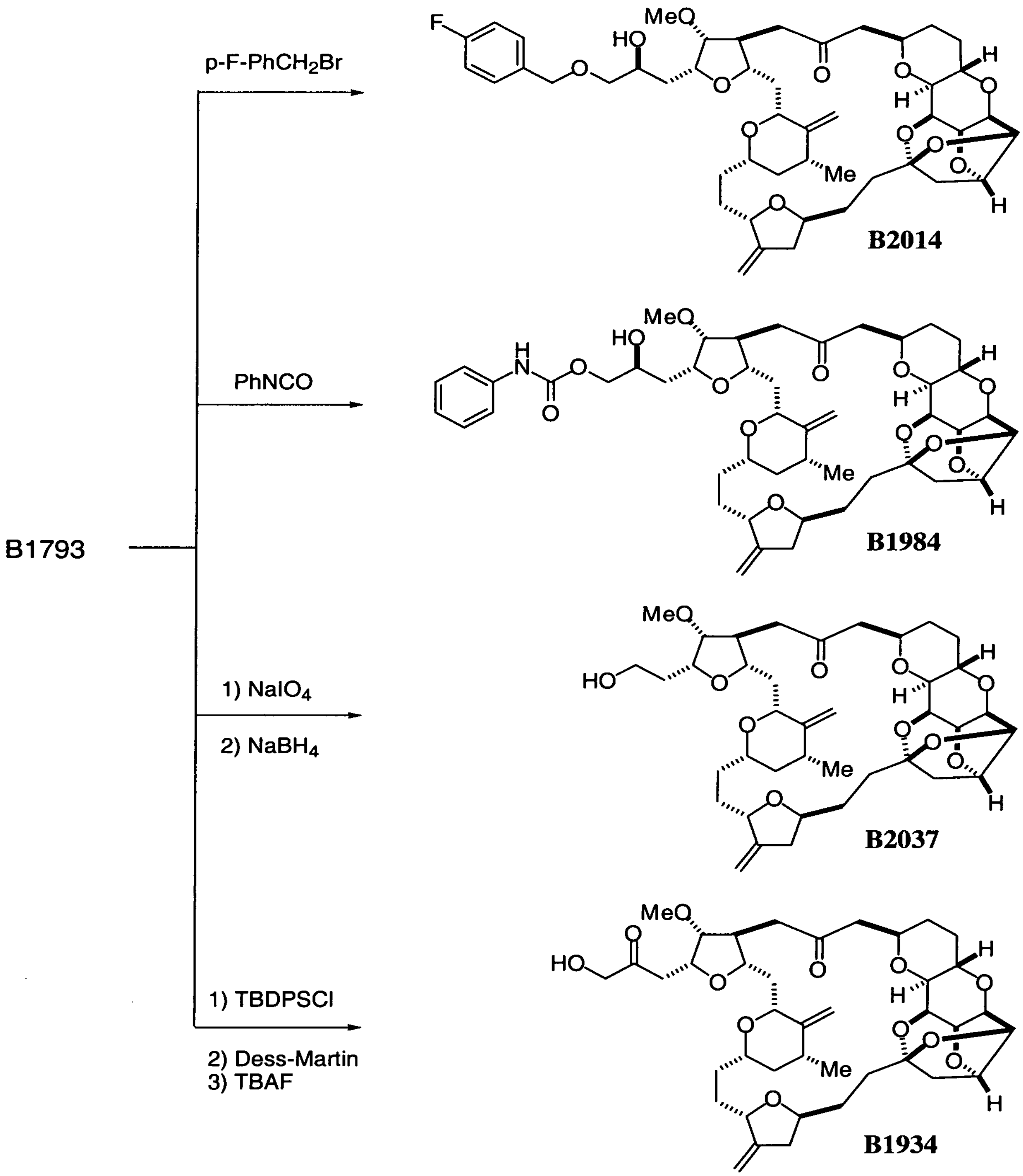
Scheme 7



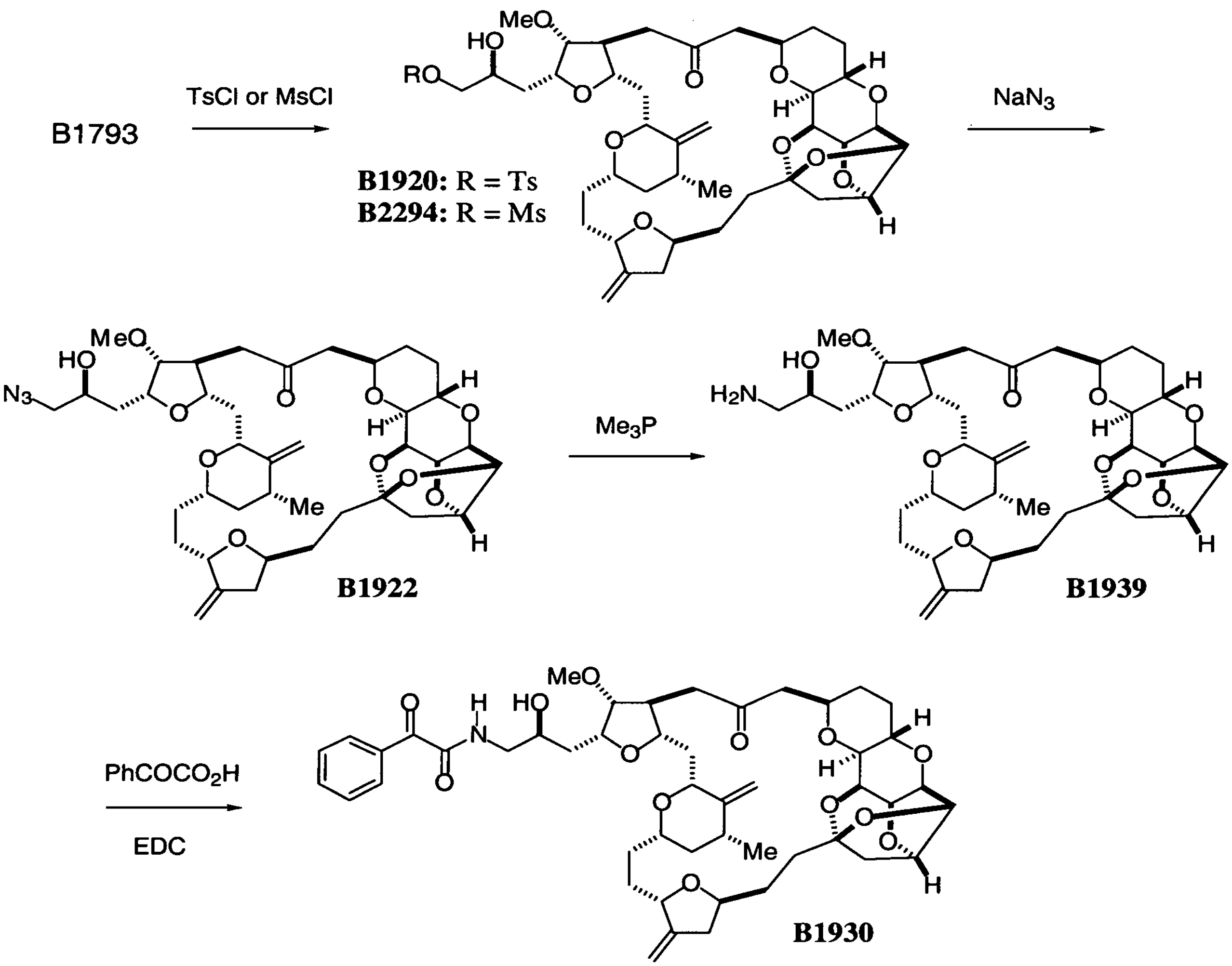
B2318

204

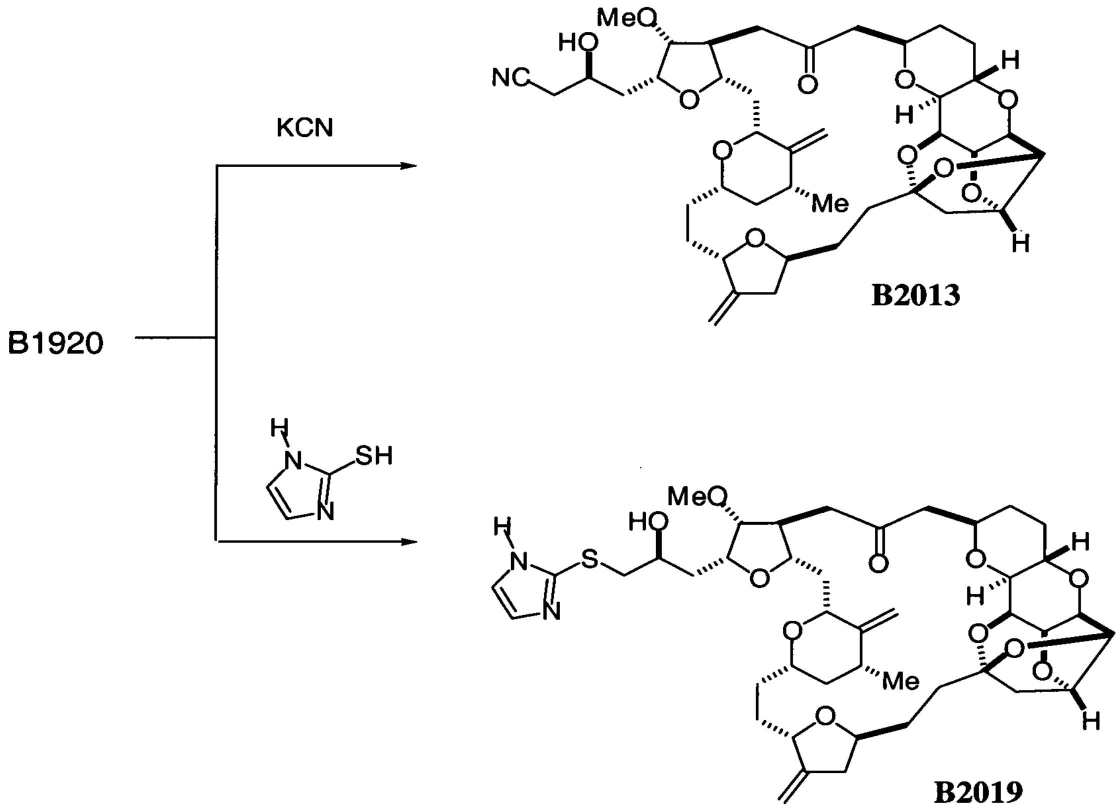
Scheme 8


B1793
p-F-PhCH2Br
PhNCO
1) NaIO4
2) NaBH4
1) TBDPSCl
2) Dess-Martin
3) TBAF
B2014
B1984
B2037
B1934

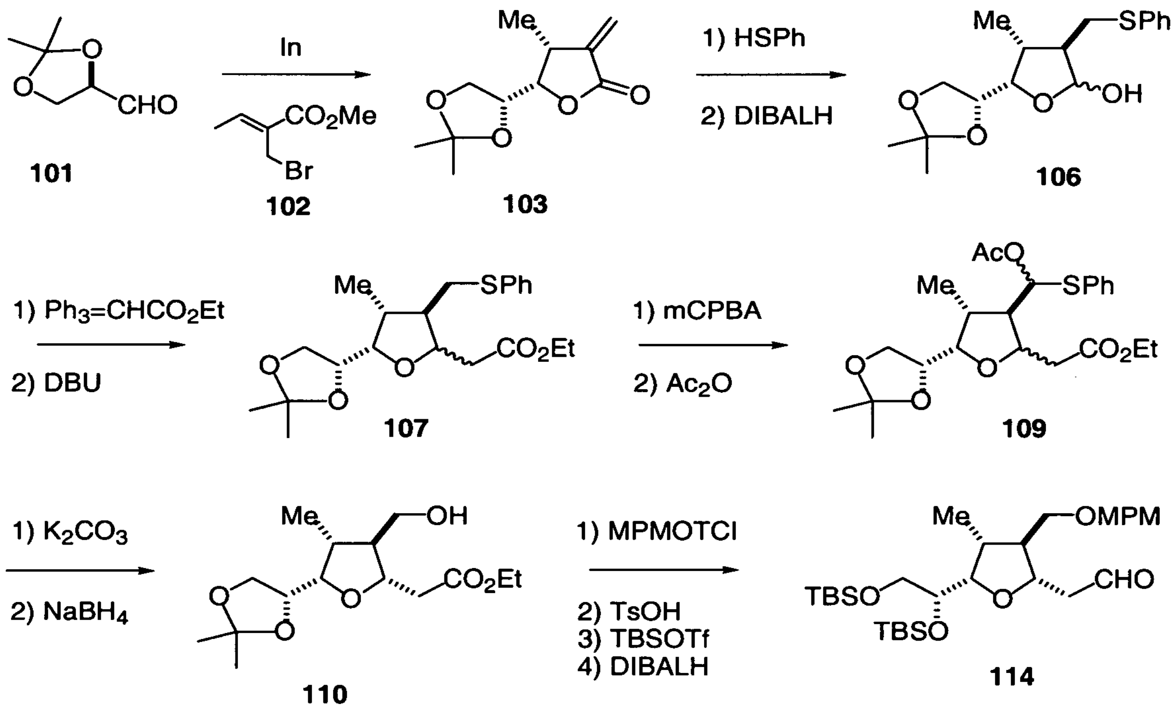
Scheme 9


B1793
TsCl or MsCl
B1920: R = Ts
B2294: R = Ms
NaN3
B1922
Me3P
B1939
PhCOCO2H
EDC
B1930

Scheme 10

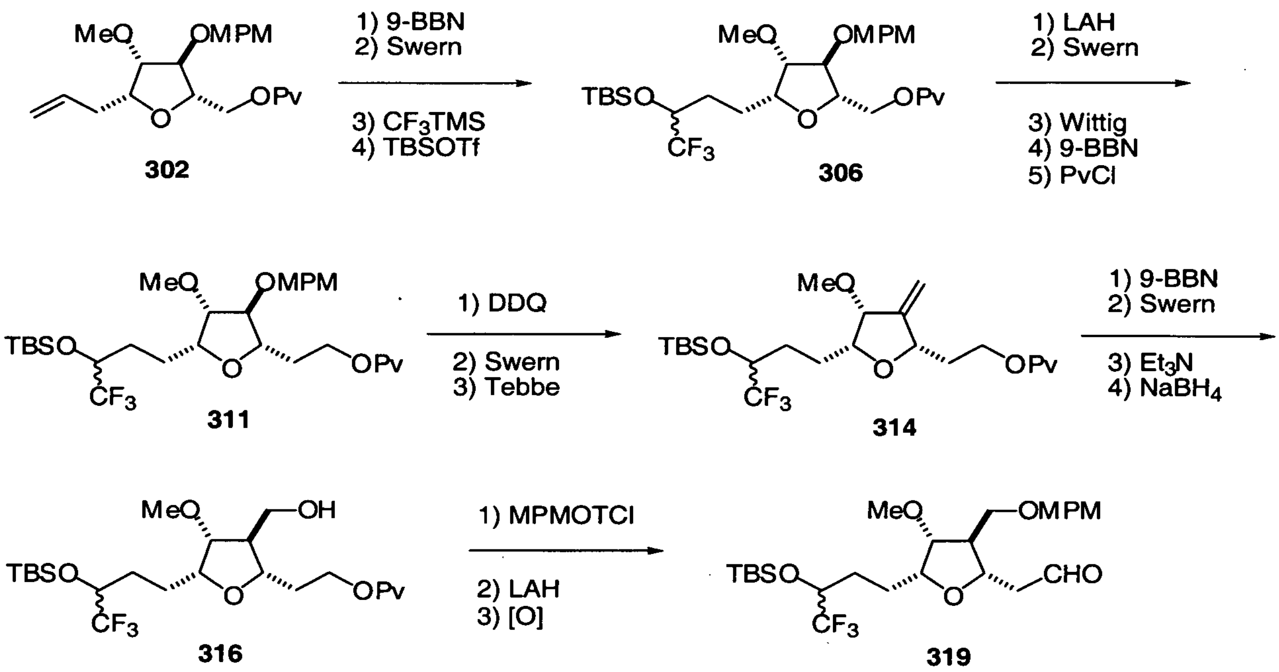


Scheme 11

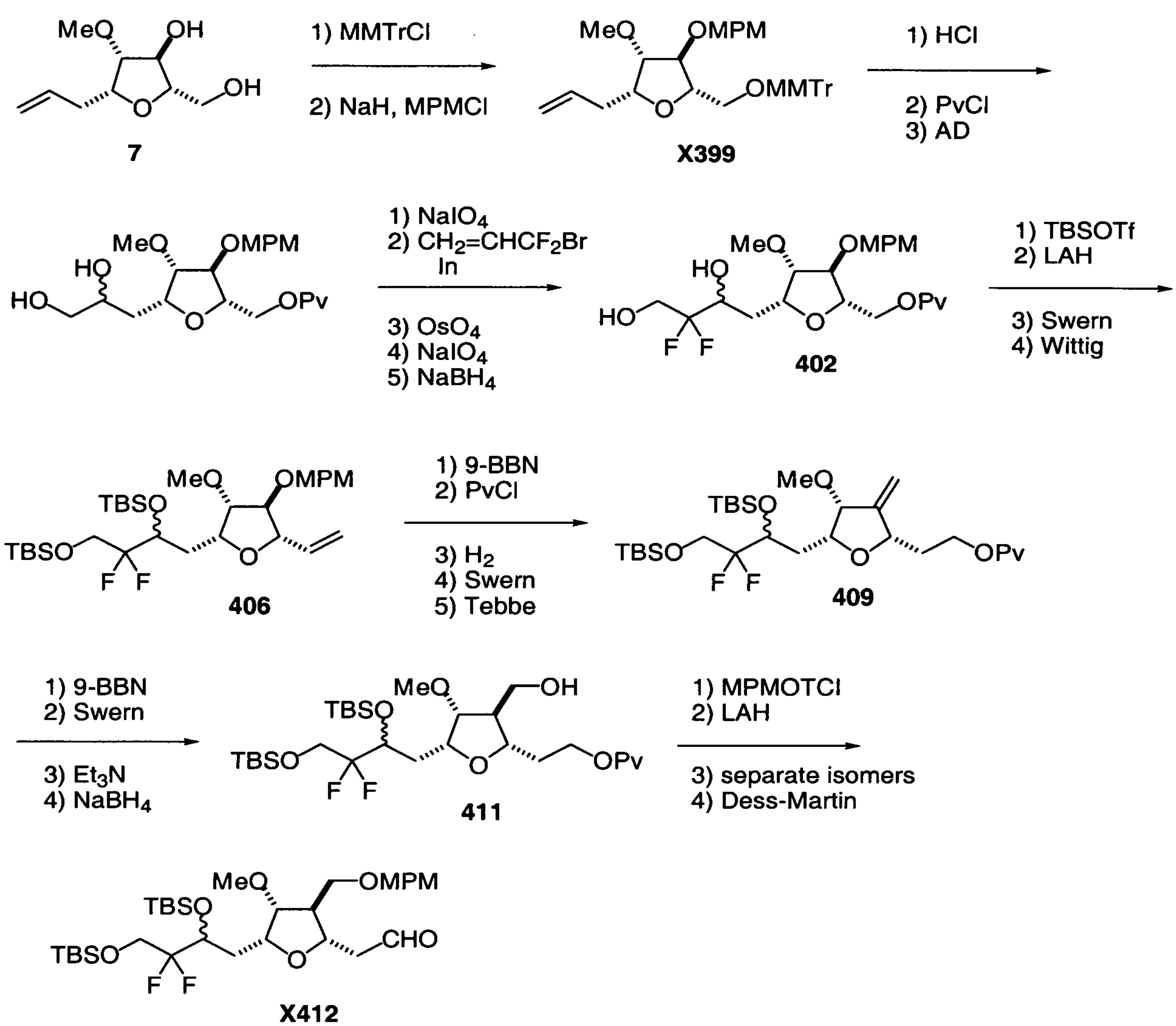


In
CO2Me
Br
101
102
103
Me
1) HSPh
2) DIBALH
SPh
OH
106
1) Ph3=CHCO2Et
2) DBU
CO2Et
107
1) mCPBA
2) Ac2O
AcO
109
1) K2CO3
2) NaBH4
110
1) MPMOTCl
2) TsOH
3) TBSOTf
4) DIBALH
OMPM
CHO
TBSO
114

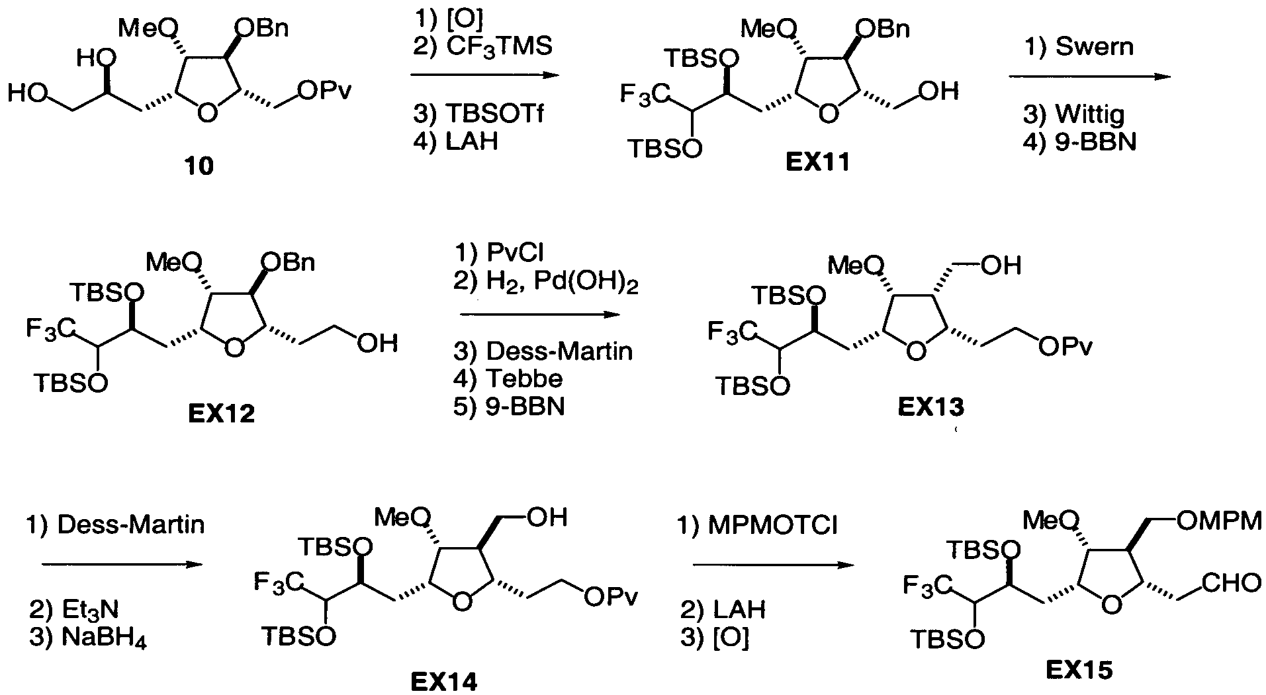
Scheme 12


MeO
OMPM
OPv
302
1) 9-BBN
2) Swern
3) CF3TMS
4) TBSOTf
TBSO
CF3
306
1) LAH
2) Swern
3) Wittig
4) 9-BBN
5) PvCl
311
1) DDQ
2) Swern
3) Tebbe
314
1) 9-BBN
2) Swern
3) Et3N
4) NaBH4
OH
316
1) MPMOTCl
2) LAH
3) [O]
CHO
319

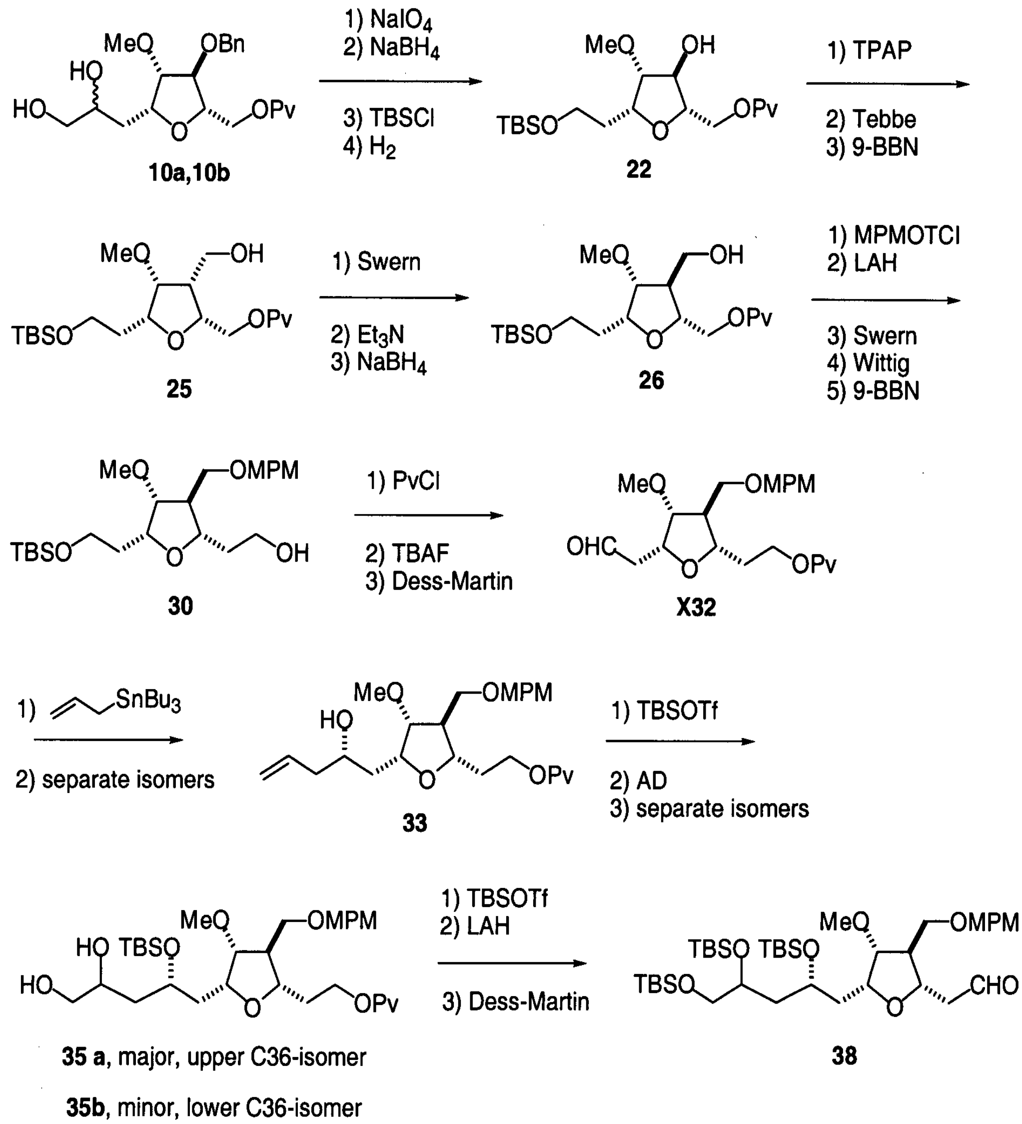
Scheme 13


MeO
OH
OH
7
1) MMTrCl
2) NaH, MPMCl
OMPM
OMMTr
X399
1) HCl
2) PvCl
3) AD
HO
OPv
1) $NaIO_4$
2) CH_2=$CHCF_2Br$
In
3) OsO_4
4) $NaIO_4$
5) $NaBH_4$
F F
402
1) TBSOTf
2) LAH
3) Swern
4) Wittig
TBSO
406
1) 9-BBN
2) PvCl
3) H_2
4) Swern
5) Tebbe
409
1) 9-BBN
2) Swern
3) Et_3N
4) $NaBH_4$
411
1) MPMOTCl
2) LAH
3) separate isomers
4) Dess-Martin
CHO
X412

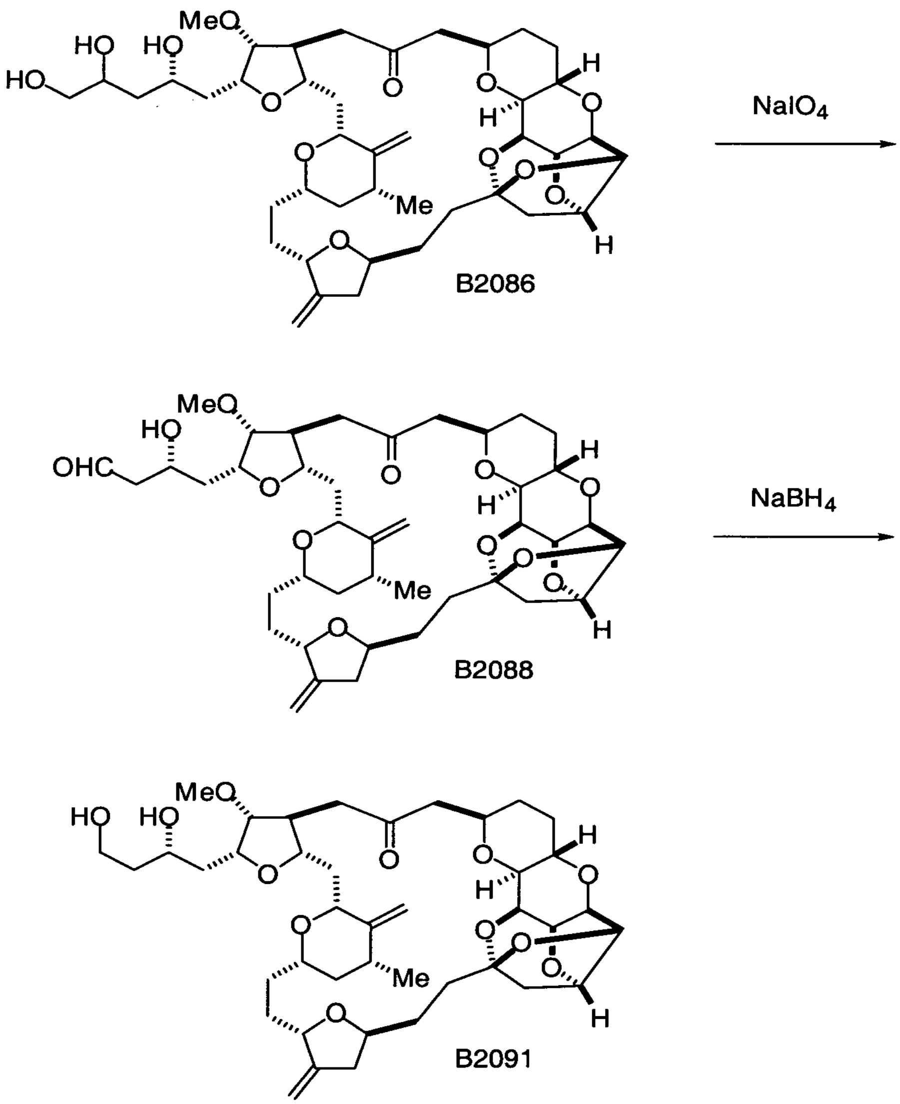
Scheme 14


MeO
OBn
HO
HO
OPv
10
1) [O]
2) CF_3TMS
3) TBSOTf
4) LAH
TBSO
F_3C
TBSO
OH
EX11
1) Swern
3) Wittig
4) 9-BBN
EX12
1) PvCl
2) H_2, $Pd(OH)_2$
3) Dess-Martin
4) Tebbe
5) 9-BBN
EX13
1) Dess-Martin
2) Et_3N
3) $NaBH_4$
EX14
1) MPMOTCl
2) LAH
3) [O]
OMPM
CHO
EX15

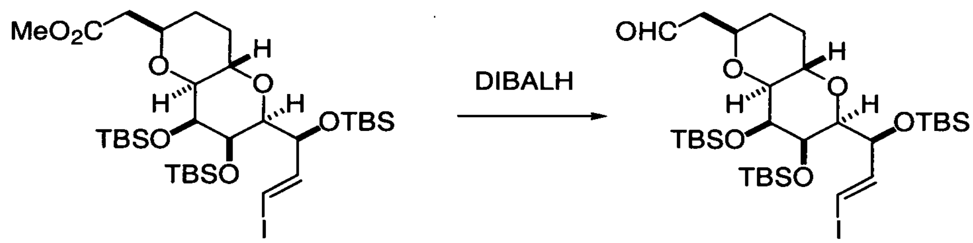
Scheme 15


MeO
OBn
HO
HO
OPv
10a,10b
1) NaIO4
2) NaBH4
3) TBSCl
4) H2
MeO
OH
TBSO
OPv
22
1) TPAP
2) Tebbe
3) 9-BBN
MeO
OH
TBSO
OPv
25
1) Swern
2) Et3N
3) NaBH4
MeO
OH
TBSO
OPv
26
1) MPMOTCl
2) LAH
3) Swern
4) Wittig
5) 9-BBN
MeO
OMPM
TBSO
OH
30
1) PvCl
2) TBAF
3) Dess-Martin
MeO
OMPM
OHC
OPv
X32
1) SnBu3
2) separate isomers
MeO
OMPM
HO
OPv
33
1) TBSOTf
2) AD
3) separate isomers
MeO
OMPM
HO TBSO
HO
OPv
35 a, major, upper C36-isomer
35b, minor, lower C36-isomer
1) TBSOTf
2) LAH
3) Dess-Martin
MeO
OMPM
TBSO TBSO
TBSO
CHO
38

Scheme 16


MeO
HO
HO
HO
O
O
O
H
H
O
O
O
O
Me
O
H
B2086
NaIO4
MeO
HO
OHC
B2088
NaBH4
MeO
HO
HO
B2091

Synthesis of Key Fragment F-3:



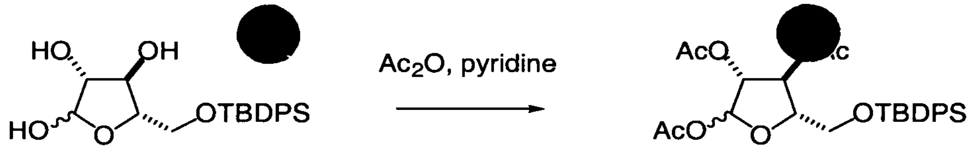
XF-3 **F-3**

Key Fragment F-3. DIBALH (1 M in toluene, 3.86 mL) was added to a solution of **XF-3** (1,46 g, 1.93 mmol) in toluene (37 mL) at -78 °C. After stirring for 10 min, the reaction was quenched by careful addition of MeOH (0.46 mL) and H_2O (0.21 mL), warmed to rt and stirred for 15 min. The white suspension was filtered through Celite with 1:1 CH_2Cl_2/Et_2O. The filtrate was concentrated and purified by column chromatography (10% EtOAc-hexanes) to give key fragment **F-3** (1.34 g, 96%) as an oil.

Synthesis of B1793:

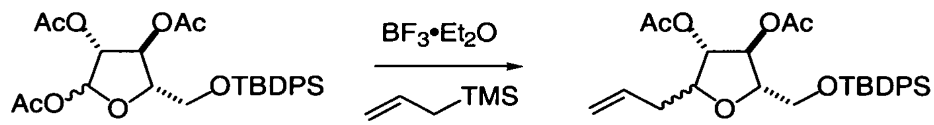
L-arabinose **1**

Triol 1 A solution of TBDPSCl (444 mL, 1.7 mol) in DMF (0.5 L) was added in three portions to a suspension of L-arabinose (250.0 g, 1.66 mol), imidazole (231.4 g, 3.40 mol) and DMF (2.5 L). The addition of each portion took 1.5 h with a 30 min and a 15 h interval separating the second and third portions, respectively. The resulting solution was stirred for 3 h, concentrated and purified by flash chromatography (5% to 33% EtOAc-hexanes) to provide triol **1** (394 g, 61%).



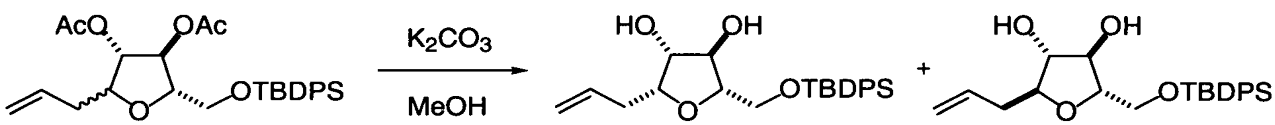
1 2

Triacetate 2 Acetic anhydride (6.06 mol) was added over 1.5 h to triol **1** (1.01 mol) in pyridine (1.0 L) at 15 °C. The solution was stirred for 1 h, concentrated and purified by flash chromatography (15% to 25% EtOAc-hexanes) to afford triacetate **2** (518 g, 97%).



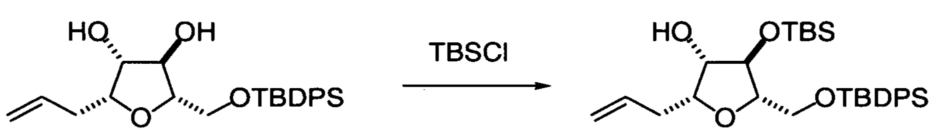
2 3

Diacetates 3 Allyltrimethylsilane (1.11 mol) followed by $BF_3 \bullet OEt_2$ (1.11 mmol) was added over 1.5 h to triacetate **2** (164 g, 0.32 mol) in toluene (1.5 L) at 0 °C. The orange solution was stirred for 1 h at 0 °C and for 2 h at rt. The mixture was slowly poured into saturated aqueous $NaHCO_3$ (1.7 L) at 0 °C and stirred for 30 min. The separated aqueous layer was extracted with EtOAc (3 × 600 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (5% to 10% EtOAc-hexanes) to furnish a mixture of diacetates **3** (108 g, 69%).



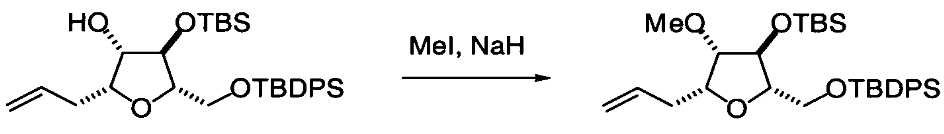
3 4a 4b

Diol 4a Solid K_2CO_3 (72 mmol) was added to diacetates **3** (108 g, 218 mmol) in MeOH (0.5 L) at rt. The suspension was stirred for 2.5 h and then concentrated. The orange residue was suspended in saturated aqueous NH_4Cl (150 mL), extracted with EtOAc (3 × 150 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (15% to 50% EtOAc-hexanes) to afford alpha-isomer **4a** (33.86 g, 37%), and beta-isomer **4b** (58 g, 63%).



4a 5

Alcohol 5 Imidazole (16.75 g, 246 mmol) and TBSCl (16.08 g, 107 mmol) were added to a solution of diol **4a** (33.86 g, 82 mmol) in CH_2Cl_2 (250 mL) at 0 °C. After 18 h at 0 °C and 5 h at rt, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (250 mL), stirred for 30 min and the layers were allowed to separate. The aqueous layer was extracted with EtOAc (3 × 250 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (2% to 50% EtOAc-hexanes) to furnish alcohol **5** (36.0 g, 83%).

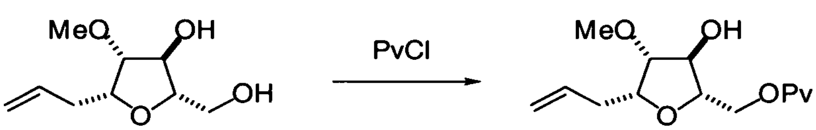


5 **6**

Methyl ether 6 Iodomethane (16.5 mL, 265 mmol) and NaH (60% in mineral oil, 5.28 g, 132 mmol) were added to a solution of alcohol **5** (34.93 g, 66 mmol), THF (320 mL) and DMF (80 mL) at 0 °C. After 19 h at 0 °C, the reaction was quenched with saturated aqueous NH_4Cl and saturated aqueous $Na_2S_2O_3$. The resulting mixture was stirred for 20 min and the layers were allowed to separate. The aqueous phase was extracted with EtOAc (3 × 200 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (3% EtOAc-hexanes) to afford methyl ether **6** (34.23 g, 96%).

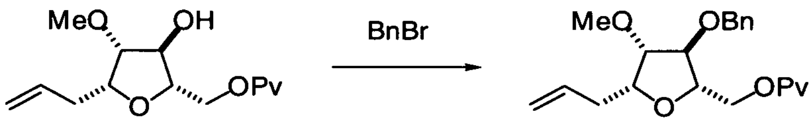
6 **7**

Diol 7 HCl (37% aqueous solution, 12.75 mL, 153 mmol) was added to a solution of methyl ether **6** (32.93 g, 61 mmol) in MeOH (110 mL) at rt. After 17 h, $NaHCO_3$ (17 g) was added to the reaction mixture. The mixture was stirred for 30 min, concentrated, suspended in EtOAc and filtered. The filtrate was concentrated and purified by flash chromatography (50% EtOAc-hexanes to EtOAc) to give diol **7** (10.0 g, 87%).



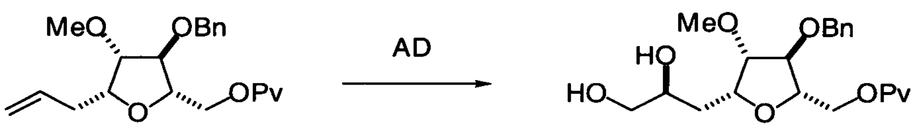
7 **8**

Alcohol 8 A solution of pivaloyl chloride (8.4 mL, 67 mmol) in pyridine (50 mL) was added over 1.5 h to a solution of diol **7** (12.24 g, 65 mmol) in pyridine (100 mL) at 0 °C. After 1 h at 0 °C and 18 h at rt, the mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc (3 × 800 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (50% EtOAc-hexanes) to furnish alcohol **8** (16.9 g, 96%).



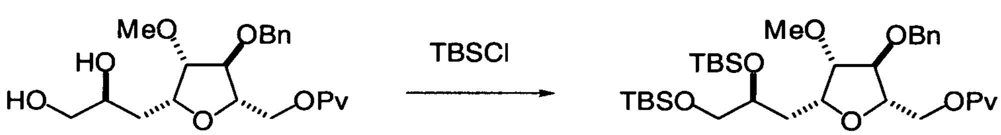
8 **9**

Olefin 9 Benzyl bromide (62 mL, 521 mmol) and Bu_4NHSO_4 (10.6 g, 31 mmol) were added to a solution of alcohol **8** (16.9 g, 62 mmol) in CH_2Cl_2 (100 mL) at 0 °C. A solution of NaOH (9.95 g, 248 mmol) in H_2O (10 mL) was added to the reaction mixture over 15 min. After 30 min at 0 °C and 18 h at rt, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (hexanes to 30% EtOAc-hexanes) to afford olefin **9** (22.1 g, 98%).



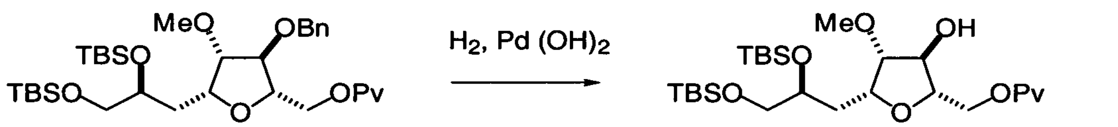
9 **10**

Diol 10 OsO_4 (0.1 M solution in toluene, 7.3 mL, 0.73 mmol) and a solution of olefin **9** (24.9 g, 69 mmol) in t-BuOH (165 mL) were added to a solution of K_2CO_3 (31.2 g, 161 mmol), $K_3Fe(CN)_6$ (74.4 g, 161 mmol), $(DHQ)_2PYR$ (1.33 g, 1.50 mmol), H_2O (500 mL) and t-BuOH (330 mL) at 0 °C. After 3 h at 0 °C, $Na_2S_2O_5 \bullet 5\ H_2O$ (37.3 g, 150 mmol) was added. The reaction mixture was warmed to rt, stirred for 1 h and extracted with EtOAc (3 × 300 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (5% isopropanol- CH_2Cl_2) to provide diol **10** (17.98 g, 75%).



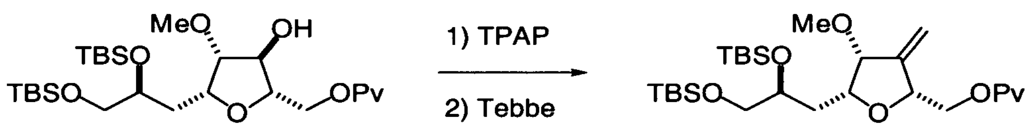
10 **11**

Silyl ether 11 Imidazole (21 g, 308 mmol) and TBSCl (26.5 g, 176 mmol) were added to a solution of diol **10** (17.4 g, 44 mmol) in DMF (90 mL) at rt. After 18 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (250 mL), stirred for 1 h and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (5% EtOAc-hexanes) to afford silyl ether **11** (25.7 g, 94%).



11 **12**

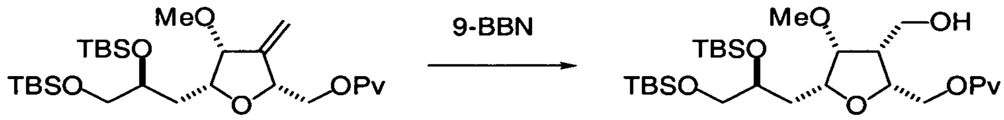
Alcohol 12 A mixture of silyl ether **11** (21.2 g, 33.8 mmol), $Pd(OH)_2$ (20%, 4.7 g, 33.8 mmol) and EtOAc (200 mL) was stirred at rt under 1 atm H_2 for 3 h. The mixture was filtered through Celite, concentrated and purified by flash chromatography (10% to 20% EtOAc-hexanes) to afford alcohol **12** (17.4 g, 96%).



12 **13**

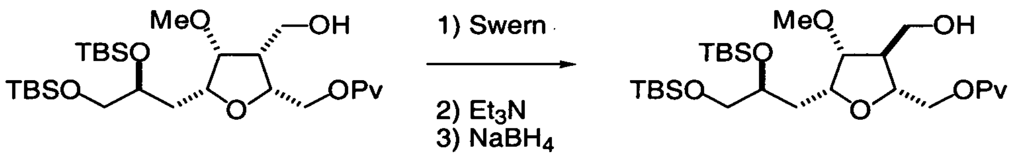
Olefin 13 4-Methylmorpholine N-oxide (7.66 g, 65 mmol) and TPAP (1.15 g, 3.26 mmol) was added in four portions over 20 min to a solution of alcohol **12** (17.4 g, 32.5 mmol) in CH_2Cl_2 (145 mL) at 0 °C. After 20 min, the reaction mixture was diluted with Et_2O (50 mL) and saturated aqueous $Na_2S_2O_5$ (50 mL) and filtered through Celite. The organic layer was separated, washed sequentially with saturated aqueous $CuSO_4$-brine (1:1) and brine, dried over Na_2SO_4, filtered through Celite, and concentrated to afford the desired crude ketone.

Tebbe reagent was prepared by stirring bis(cyclopentadienyl)titanium (11.36 g, 45.6 mmol) and Me_3Al (2.0 M in toluene, 45.6 mL, 91.2 mmol) for 4 days at rt. This material was cooled to -25 °C and a solution of crude ketone in THF (150 mL) was added. The reaction mixture was warmed to 0 °C, stirred for 30 min, quenched by slow addition of 0.1 N NaOH (3.5 mL), and then stirred for an additional 20 min at rt. The mixture was diluted with Et_2O, filtered through Celite and concentrated. The residue was dissolved in CH_2Cl_2, filtered through basic Al_2O_3, concentrated and purified by flash chromatography (5% EtOAc-hexanes) to give olefin **13** (12.8 g, 74% for two steps).



13 14

Alcohol 14 9-BBN (0.5 M in THF, 165 mL, 83 mmol) was added to a solution of olefin **13** (12.78 g, 24 mmol) in THF (280 mL) at 0 °C. After stirring for 5 h at rt, the reaction mixture was recooled to 0 °C at which time H_2O (200 mL), THF (100 mL) and $NaBO_3 \cdot 4\ H_2O$ (75 g) were added. The mixture was warmed to rt, stirred for 16 h and then concentrated. The aqueous residue was extracted with EtOAc (4 × 300 mL) and the combined organic layers were dried over Na_2SO_4. Concentration and purification by flash chromatography (20% to 35% EtOAc-hexanes) afforded alcohol **14** (12.05 g, 91%).

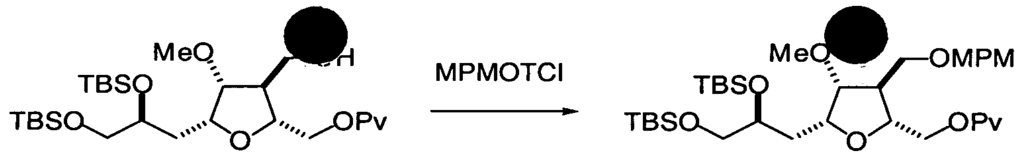


14 15

Alcohol 15 DMSO (9 mL, 127 mmol) was added to a solution of oxalyl chloride (5.6 mL, 64 mmol) in CH_2Cl_2 (350 mL) at -78 °C. After stirring for 15 min, a solution of alcohol **14** (11.7 g, 0.021 mmol) in CH_2Cl_2 (50 mL) was added and stirring was continued for 1 h, after which Et_3N (26.7 mL, 192 mmol) was added. The reaction mixture was warmed to 0 °C, stirred for 15 min, diluted with saturated aqueous NH_4Cl, and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to furnish the desired crude aldehyde.

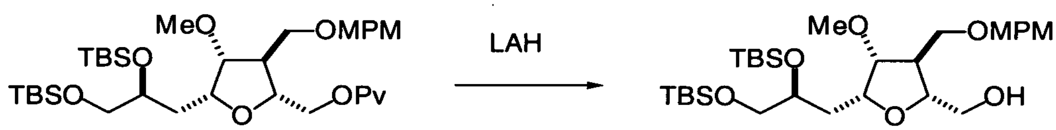
This material was dissolved in CH_2Cl_2 (200 mL) and treated with Et_3N (20 mL) at rt. After stirring overnight, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were dried over Na_2SO_4, concentrated and filtered through a short SiO_2 column (20% EtOAc-hexanes) to afford the crude epimerized product.

The aldehyde was dissolved in Et_2O-EtOH (1:1, 100 mL), cooled to 0 °C and treated with sodium borohydride (1.21 g, 32 mmol). The mixture was stirred for 20 min, carefully diluted with saturated aqueous NH_4Cl, stirred for 30 min at rt and extracted with CH_2Cl_2 (3 × 150 mL). The combined extracts were dried over Na_2SO_4, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to afford alcohol **15** (9.95 g, 85% for three steps).



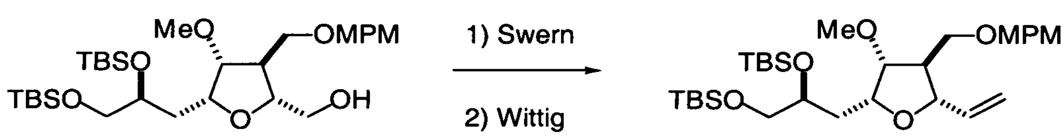
15 **16**

MPM-ether 16 BF_3•OEt_2 (0.1 M in CH_2Cl_2, 1.8 mL, 0.18 mmol) was added to a solution of alcohol **15** (9.87 g, 18 mmol), MPM-trichloroimidate (4.9 mL, 27 mmol) and CH_2Cl_2 (175 mL) at 0 °C. After 40 min, a second portion of BF_3•OEt_2 (0.1 M in CH_2Cl_2, 0.9 mL, 0.09 mmol) was added to the reaction mixture. After 20 min, the reaction was quenched with saturated aqueous NH_4Cl, stirred for 1 h at rt and diluted with Et_2O (600 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (150 mL). The combined organic extracts were washed sequentially with 0.1 N aqueous NaOH, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to give MPM-ether **16** (10.20 g, 85%).



16 **17**

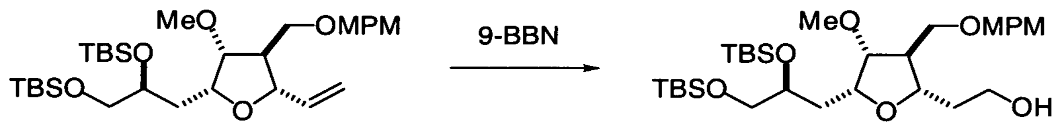
Alcohol 17 LAH (1 M in THF, 22.5 mL, 22.5 mmol) was added to a solution of MPM-ether **16** (10.05 g, 15 mmol) in Et_2O (1.0 L) at 0 °C. After 30 min, the reaction was cautiously quenched with H_2O (1.3 mL), and 1 N aqueous NaOH (1.3 mL). After stirring for 1 h at rt, the suspension was filtered through Celite, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to afford alcohol **17** (8.18 g, 93%).



17 **18**

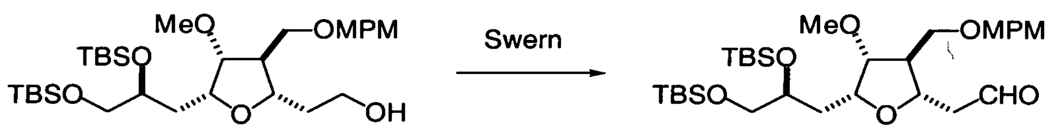
Olefin 18 DMSO (5.8 mL, 82.4 mmol) was added to a solution of oxalyl chloride (3.6 mL, 41.2 mmol) in CH_2Cl_2 (100 mL) at -78 °C. After 15 min, a solution of alcohol **17** (7.94 g, 13.5 mmol) in CH_2Cl_2 (35 mL) was added to the reaction mixture. After stirring for 1 h, Et_3N (17 mL, 122 mmol) was added, the mixture was warmed to 0 °C, stirred for 20 min, diluted with saturated aqueous NH_4Cl and then extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, concentrated and filtered through a short SiO_2 column (20% EtOAc-hexanes) to furnish the desired crude aldehyde.

n-BuLi (1.6 M, 20 mL, 30 mmol) was added dropwise to a solution of CH_3PPh_3Br (10.1 g, 30 mmol) in THF (350 mL) and DMSO (100 mL) at 0 °C. After 1 h, a solution of the crude aldehyde in THF (50 mL) was added. The reaction mixture was warmed to rt and stirred for 3 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with EtOAc (3 × 500 mL). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (7% EtOAc-hexanes) to afford olefin **18** (5.57 g, 71% yield for 2 steps).



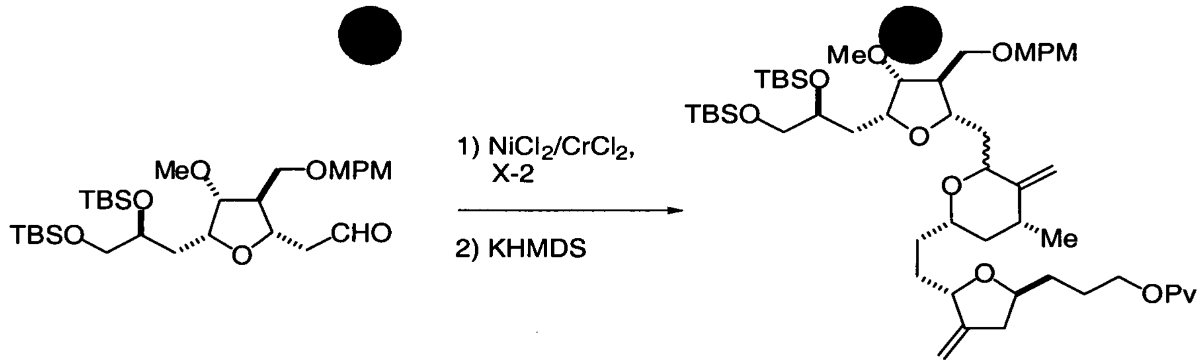
18 **19**

Alcohol 19 9-BBN (0.5 M in THF, 65 mL, 33 mmol) was added to a solution of olefin **18** (5.56 g, 9.6 mmol) in THF (85 mL) at 0 °C. The mixture was stirred for 5 h at rt and then recooled to 0 °C. H_2O (200 mL), THF (100 mL), and $NaBO_3$•4 H_2O (30 g) were sequentially added. After stirring overnight at rt, the organic volatiles were removed under reduced pressure. The aqueous residue was extracted with EtOAc (3 × 200 mL) and the combined organic layers were dried over Na_2SO_4. Concentration and purification by flash chromatography (30% EtOAc-hexanes) afforded alcohol **19** (12.05 g, 92%).



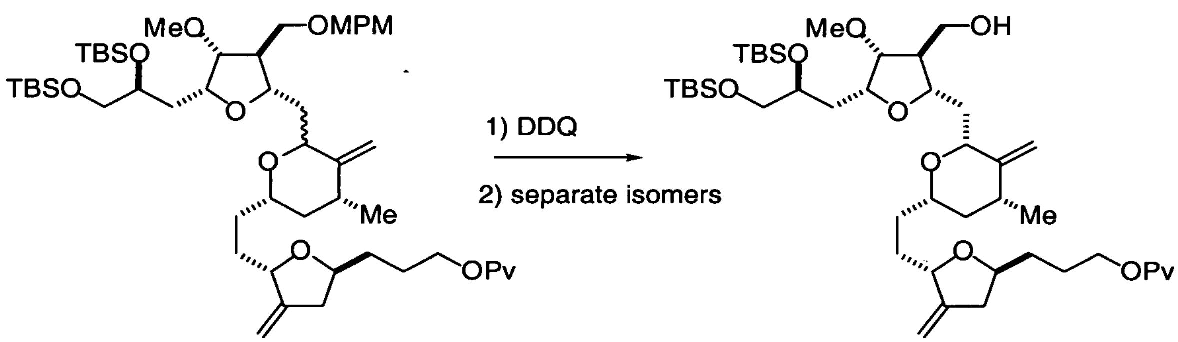
19 **20**

Aldehyde 20. DMSO (1.36 mL, 19.2 mmol) was added dropwise over 4 min to a solution of oxalyl chloride (1.26 mL, 14.4 mmol) in CH_2Cl_2 (120 mL) at -78 °C. After stirring for 10 min, a solution of alcohol **19** (5.76 g, 9.61 mmol) in CH_2Cl_2 (20 mL) was added via cannula. The transfer was completed by rinsing with additional CH_2Cl_2 (2 × 5 mL). After stirring for 20 min, the mixture was treated with Et_3N (5.36 mL, 38.4 mmol) and stirred for 10 min at -78 °C, 30 min at 0 °C and 10 min at rt. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (200 mL) and the separated aqueous layer was extracted with CH_2Cl_2 (3×) followed by EtOAc (100 mL). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to furnish aldehyde compound **20** (5.28 g, 92%) as an oil.



20 **B2318**

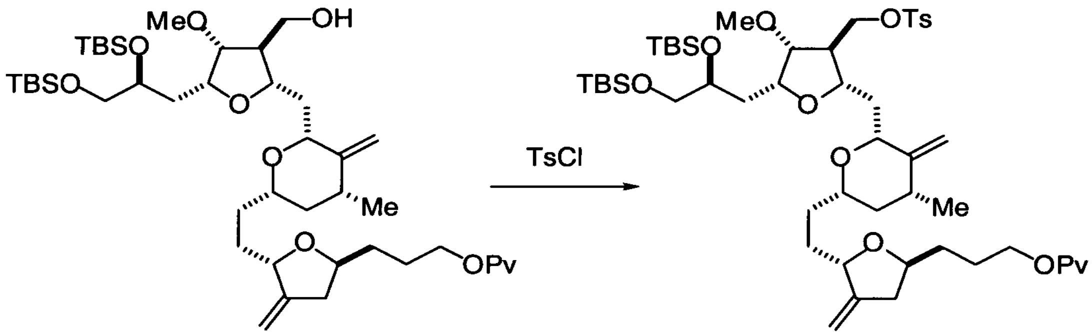
B2318. 0.1% $NiCl_2/CrCl_2$ (w/w, 3.21 g) and 1% $NiCl_2/CrCl_2$ (w/w, 4.31 g) was added to a solution of aldehyde **20** (3.73 g, 6.25 mmol), key fragment **F-2** exemplified by vinyl iodide **X2**. (5.10 g, 9.16 mmol), THF (85 mL) and DMF (21 mL) at rt in a glove box. The reaction mixture was stirred for 24 h, removed from the glove box, cooled to 0 °C, diluted with EtOAc (100 mL), quenched with saturated NH_4Cl (200 mL) and stirred for 30 min. The separated aqueous phase was extracted with EtOAc (6×) and the combined organic layers were dried over Na_2SO_4 concentrated and purified by column chromatography (20% to 30%) to give **B2318** (~3 g) contaminated with close running impurities and the uncyclized intermediate (4.61 g). The latter (4.61 g, 4.48 mmol,) was dissolved in THF (150 mL), cooled to 0 °C and treated with KHMDS (0.5 M in toluene, 14 mL, 7.0 mmol) over a 2 min period. After stirring at 0 °C for 15 min, the reaction was quenched with saturated aqueous NH_4Cl (150 mL) and warmed to rt. The separated aqueous layer was extracted with EtOAc (3×) and the combined organic phases were dried over Na_2SO_4, concentrated and combined with the partially purified product obtained above. Column chromatography (10% EtOAc-hexanes) afforded **B2318** (3.17 g, 55%) as an inseparable ~3:1 mixture of C27 diastereomers.



B2318 **B2317**

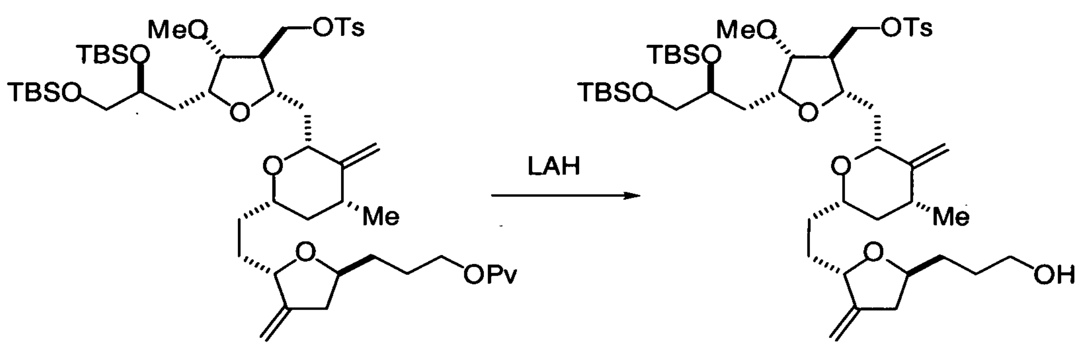
B2317. DDQ (1.45 g, 6.42 mmol) was added portion-wise over 30 min to a stirred solution of **B2318** (3.12 g, 3.35 mmol) in CH_2Cl_2 (50 mL) and pH 7 phosphate buffer (5 mL) at rt. The reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL), stirred for 5 min, diluted with additional saturated aqueous $NaHCO_3$ (100 mL), H_2O (200 mL) and extracted

with Et_2O (5×). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (15% to 30% EtOAc-hexanes) to give recovered **B2318** (1.40 g) and a mixture of the C27 isomeric products. The recovered **B2318** was resubmitted to the reaction conditions described above to afford additional product. Recovered starting material was again cycled through the deprotection conditions. All of the desired material was combined and separated by MPLC to afford **B2317** (1.65 g, 61%).



B2317 **B2316**

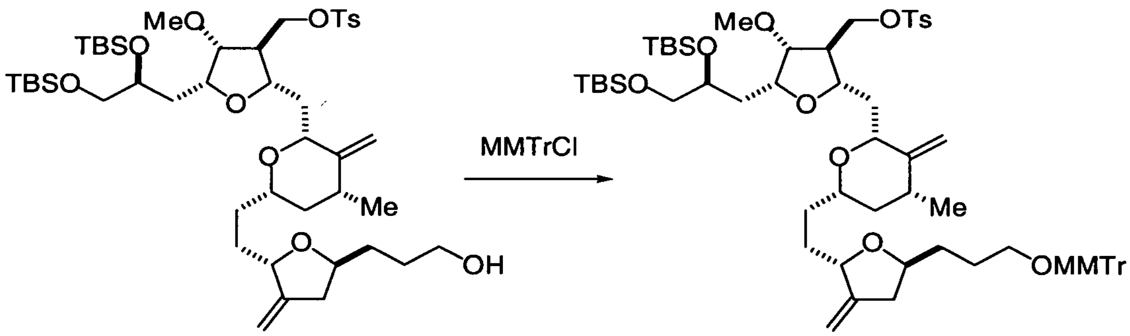
B2316. TsCl (0.63 g, 3.30 mmol) was added to a solution of **B2317** (1.60 g, 1.97 mmol) in CH_2Cl_2 (8 mL) and pyridine (2 mL) at rt. After stirring for 29 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL) and H_2O (10 mL). The separated aqueous layer was extracted with Et_2O and the combined organic layers were dried over Na_2SO_4, concentrated and purified by column chromatography (15% to 30% EtOAc-hexanes) to give **B2316** (2.01 g, 92%) as an oil along with recovered **B2317** (92 mg, 5.8%).



B2316 **B2315**

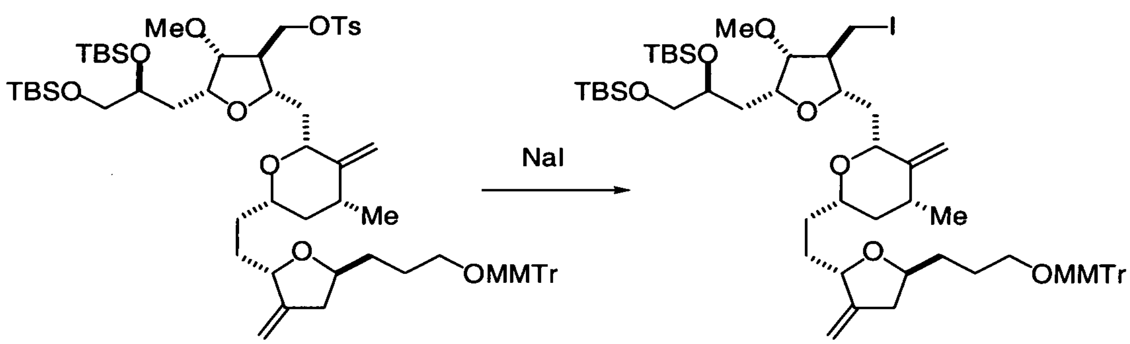
B2315. LAH (1 M in THF, 2.61 mL, 2.61 mmol) was added over 1 min to a solution of **B2316** (1.68 g, 1.74 mmol) in Et_2O (80 mL) at 0 °C. After stirring for 7 min, the reaction was quenched by careful addition of MeOH (0.42 mL, 10.4 mmol) and H_2O (0.19 mL, 10 mmol), warmed to rt and stirred for 20 min. Filtration through Celite with 1:1 CH_2Cl_2-Et_2O,

concentration and purification by column chromatography (30% to 40% EtOAc-hexanes) gave **B2315** (1.38 g, 90%) as an oil.



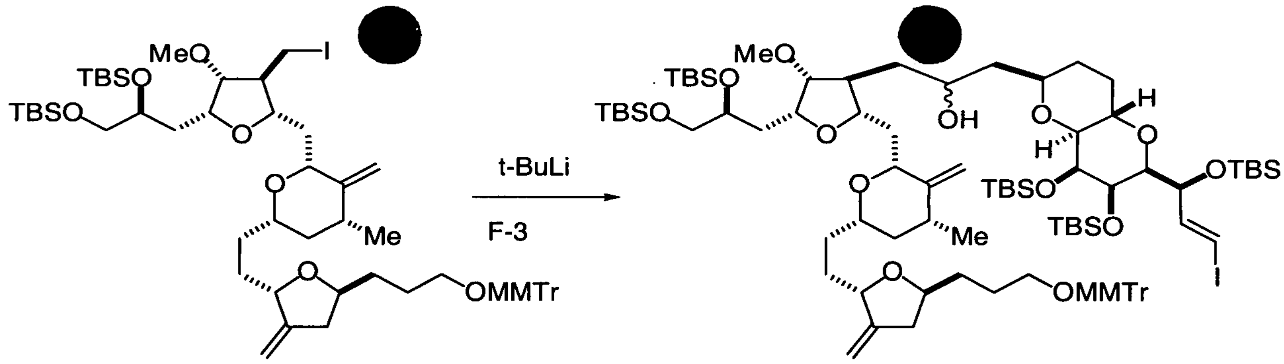
B2315 **B2314**

B2314. MMTrCl (0.70 g, 2.26 mmol) was added to a solution of **B2315** (1.33 g, 1.51 mmol) in CH_2Cl_2 (25 mL) and iPr_2NEt (0.79 mL, 4.53 mmol) at rt. The resulting mixture was stirred for 1 h and then poured into a mixture of saturated aqueous $NaHCO_3$ (20 mL), H_2O (10 mL) and Et_2O (50 mL). The separated aqueous layer was extracted with Et_2O (3×). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (CH_2Cl_2 followed by 15% to 30% EtOAc-hexanes) to give **B2314** (1.65 g, 95%) as a solid foam.



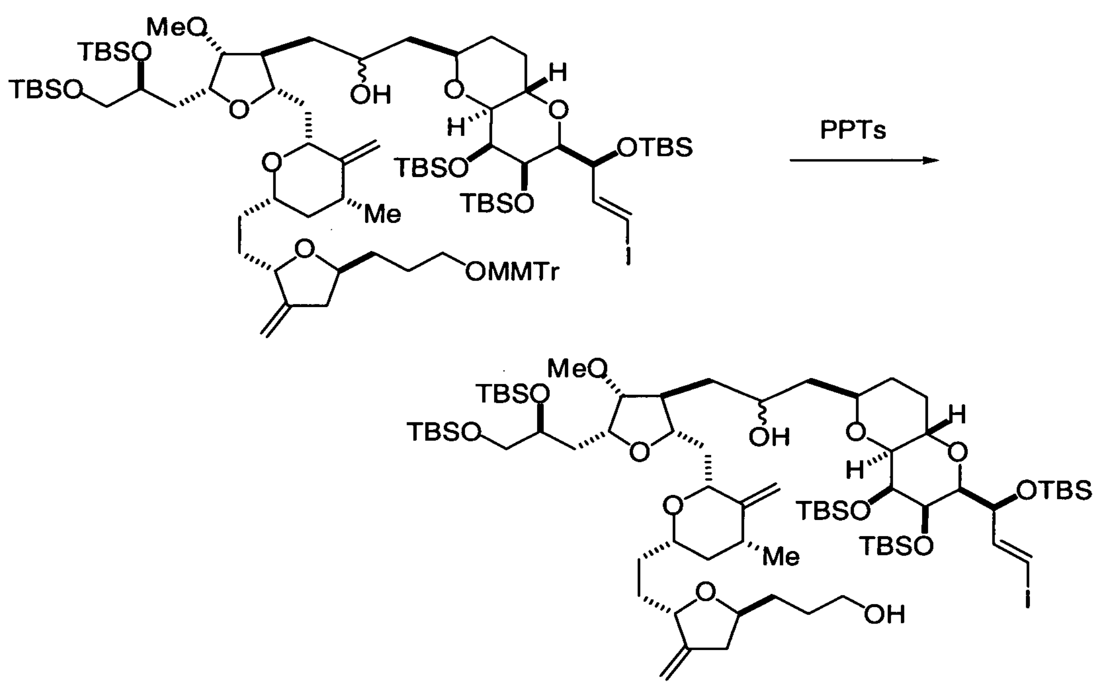
B2314 **B2313**

B2313. A mixture of **B2314** (1.60 g, 1.39 mmol) and NaI (3.10 g, 20.8 mmol) in acetone (50 mL) was heated under reflux for 13 h. After cooling to rt, the reaction mixture was diluted with EtOAc, and concentrated. H_2O (5 mL), brine (20 mL) and $Na_2S_2O_3$ (200 mg) were added and the resulting mixture was extracted with Et_2O (4×). The combined extracts were dried over Na_2SO_4, concentrated and purified by column chromatography (10% EtOAc-hexanes) to give **B2313** (1.50 g, 97%) as an oil.



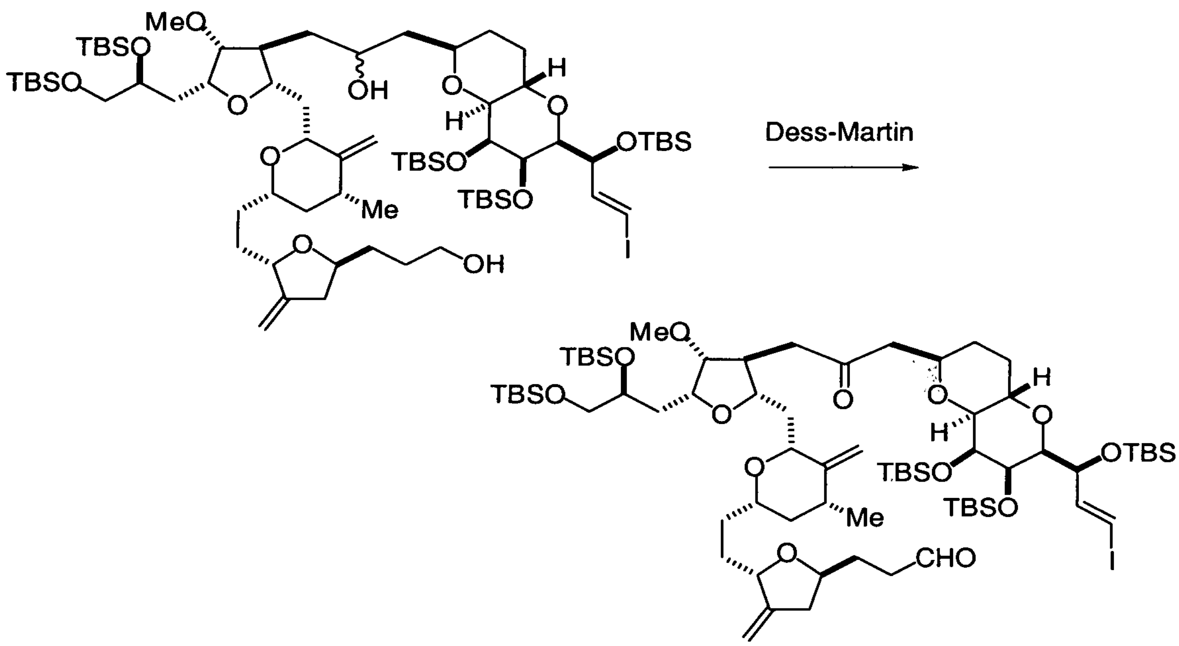
B2313 **B2307, B2308**

B2308. *Tert*-BuLi (1.7 M in pentane, 1.00 mL, 1.7 mmol) was added over 1 min to a solution of **B2313** (0.90 g, 0.81 mmol) in Et_2O (14 mL) at –78 °C. After stirring for 9 min, the mixture was transferred via cannula over 4 min to a solution of key fragment **F-3** (0.83 g, 1.14 mmol) in Et_2O (4 mL) at -78 °C. The transfer was completed by rinsing with additional Et_2O (2 mL). The resultant mixture was stirred at -78 °C for 5 min and then at 0 °C for 10 min, quenched with saturated aqueous $NaHCO_3$ (30 mL) and warmed to rt. The separated aqueous layer was extracted with Et_2O (3×) and the combined organic phases were dried over Na_2SO_4 and concentrated. The residue was combined with those of other two batches (corresponding to 0.11 g and 0.44 g of **B2313**) and purified by column chromatography (10% to 20% EtOAc-hexanes) to give a mixture of **B2307** and **B2308** (1.86 g, 83%) as a solid foam. Although the isomers could be separated by prep TLC (20% EtOAc-hexanes), they were carried forward as a mixture.



B2308 **B2305, B2306**

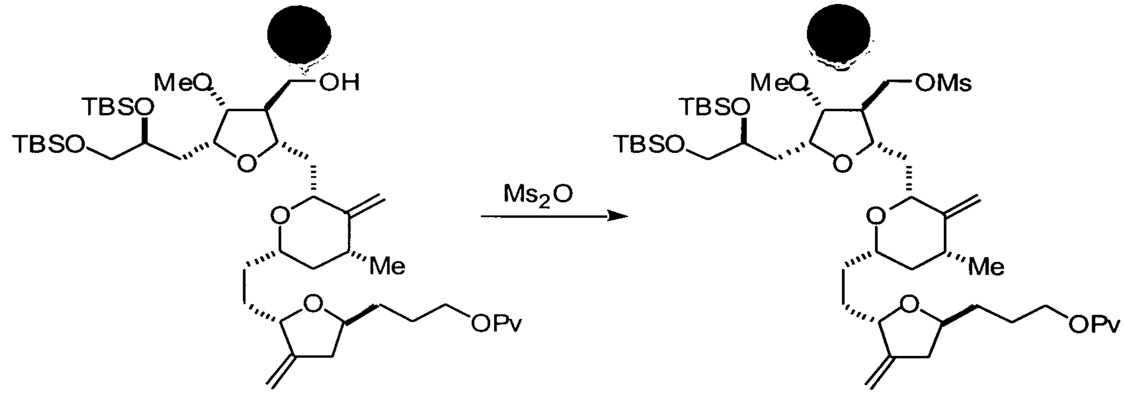
B2305 and B2306. The mixture of **B2307/B2308** (1.80 g, 1.05 mmol) was dissolved in EtOH (20 mL), treated with PPTS (10.0 mg, 0.04 mmol), stirred at rt for 11 h and then quenched with $NaHCO_3$ (20.0 mg, 0.24 mmol). After stirring for 15 min, the mixture was concentrated, azeotroped with toluene (15 mL), and purified by column chromatography (20% to 30% EtOAc-hexanes) to give a mixture of **B2305** and **B2306** (1.22 g, 81%) as a solid foam. Although the isomers could be separated by prep TLC (30% EtOAc-hexanes), they were carried forward as a mixture.



B2305, B2306 **B2304**

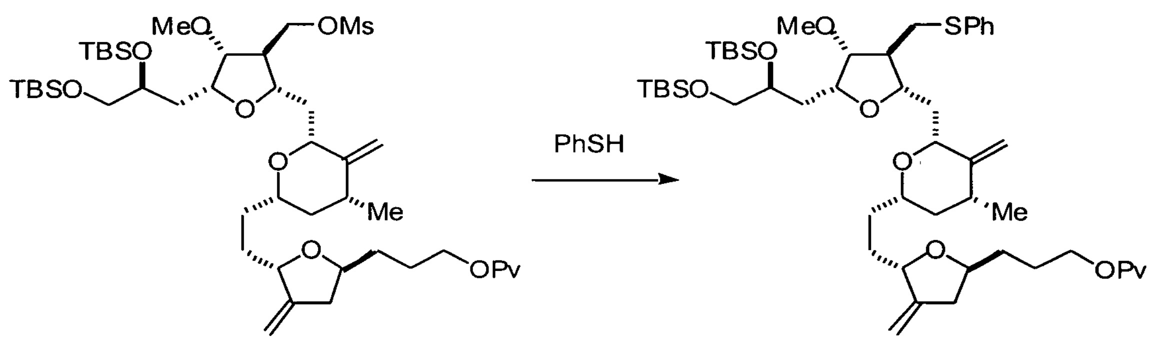
B2304. A mixture of **B2305/B2306** (1.16 g, 0.68 mmol), and Dess-Martin periodinane (0.61 g, 1.44 mmol) in CH_2Cl_2 (35 mL) was stirred at rt for 1 h. Additional Dess-Martin periodinane (0.54 g, 1.27 mmol) was added to the mixture and stirring was continued for an additional 1 h. The mixture was diluted with Et_2O (100 mL), stirred for 20 min and filtered through Celite with Et_2O. The colorless filtrate was washed with saturated aqueous $NaHCO_3$ (100 mL) and the separated aqueous layer was extracted with Et_2O (3X). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (10% to 15% EtOAc-hexanes) to give **B2304** (0.98 g, 84%) as a solid foam.

Alternatively, **B2304** may be prepared as follows and in fact the synthesis described below is superior to that given above.



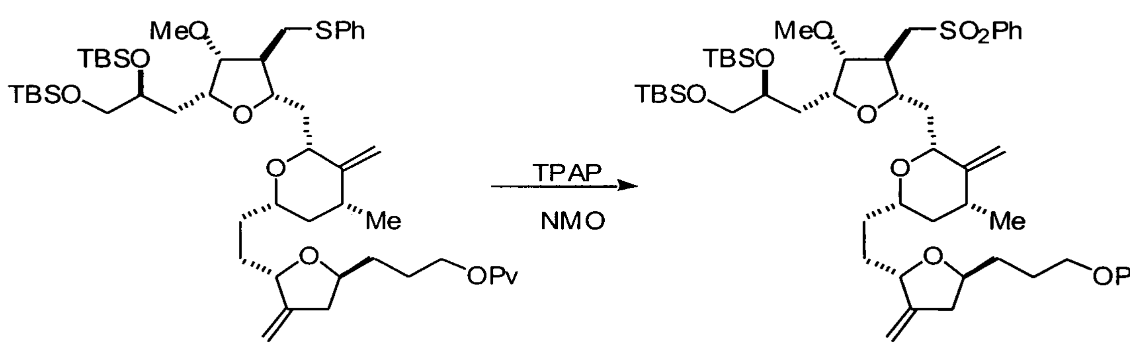
B2317 **ER804025**

To a solution of the alcohol, 2.4 g, in methylene chloride, 29 mL, was added triflic anhydride, 770 mg. The mixture was stirred for 15 minutes, extracted with saturated sodium bicarbonate, dried and chromatographed to give 2.737 g, 100%.



ER804025 **ER804026**

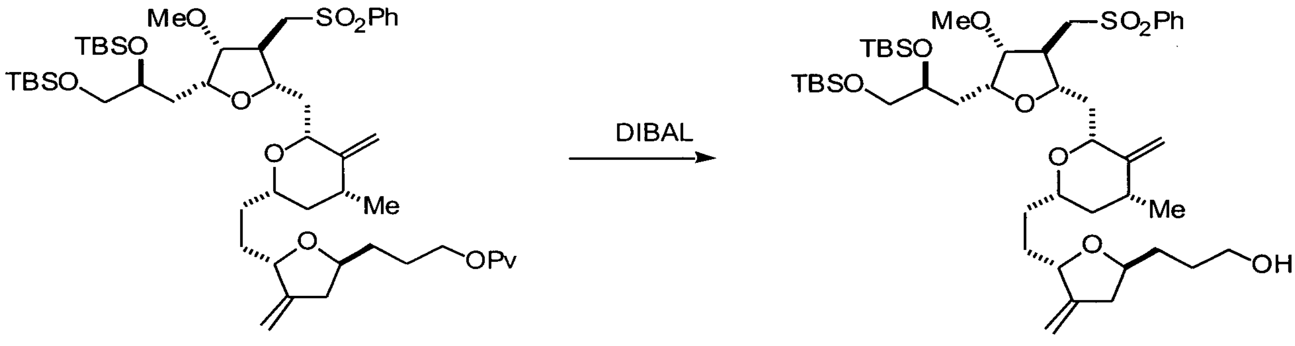
To a solution of the mesylate, 405 mg, in DMF, 0.06 mL, was added di-isopropylethylamine, 0.130 mL, followed by benzenethiol, 0.061 mL. After 4 hours and after 22 hours, additional amine, 0.03 mL, and benzenethiol, 0.015 mL, were added. After 24 hours, the mixture was diluted with 5% ethyl acetate/hexane, 1 mL and chromatographed to give 409 mg.



ER804026 **ER804027**

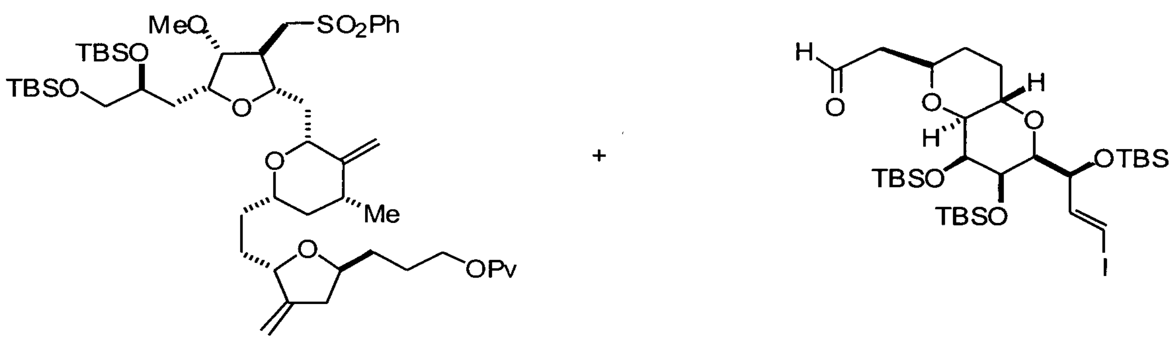
To a solution of the sulfide, 1.97 g, in acetonitrile, 16 mL, was added N-methylmorpholine oxide (NMO), and then a solution of 1.02 g, tetrapropylammonium perruthenate(VII),

(TPAP), 38 mg, in acetonitrile, 1 mL. After 3.5 hours at room temperature, the mixture was heated to 40 °C for 1 hour. The mixture was cooled and aqueous satd. Sodium thiosulfate was added and the mixture partitioned between water and ethyl acetate. The usual work-up gave 1.567 g of a brown oil.

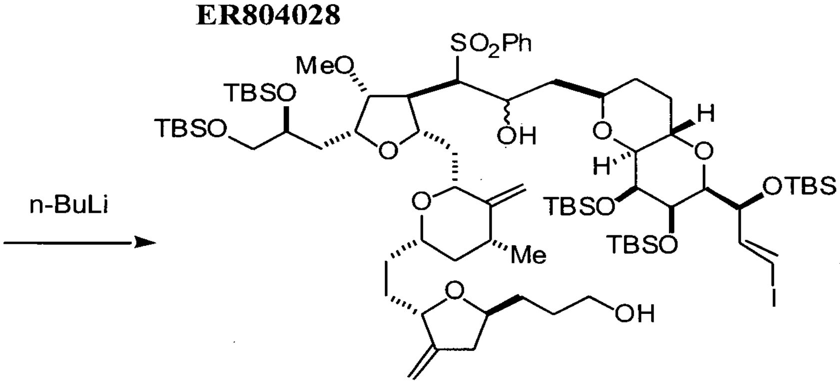


ER804027 **ER804028**

To a solution of the pivaloate ester, 1.567 g, in methylene chloride, 11.2 mL, at - 78 °C was added DIBAL, 2.5 mL of a 1 M solution in toluene. After 15 minutes, additional DIBAL, 0.8 mL, was added. After an additional 5 minutes, methanol, 0.46 mL, was slowly added followed by water, 0.2 mL. The mixture was filtered through Celite and chromatographed to give 1.386 g of an oil.



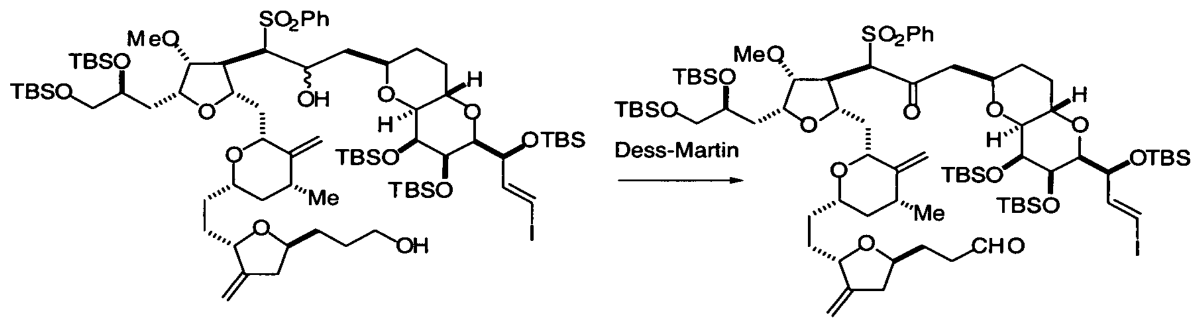
ER804028 **F-3**



ER804029

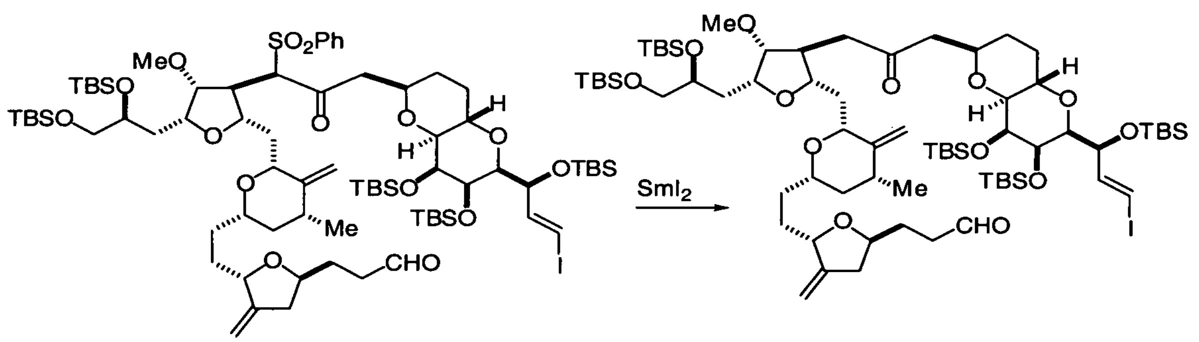
To a solution of the sulfone, 36 mg, in DME, 1 mL, at -40 ° C was added n-butyllithium, 2.8 equivalents. After 35 minutes, a solution of the aldehyde, 42 mg, in DME, 0.5 mL, was

added. After 40 minutes, saturated aqueous ammonium chloride was added and the mixture extracted with ethyl acetate. The usual work-up, followed by chromatography gave 52 mg of an oil.



ER804029 **ER804030**

To a solution of the alcohol, 42 mg, in methylene chloride, 2 mL, was added the Dess Martin reagent, 36.4 mg. The mixture was stirred for 30 minutes and ether was added. The mixture was filtered through Celite, washed with saturated sodium bicarbonate, with saturated sodium thiosulfate, worked up in the usual way and chromatographed to give 38 mg of an oil.

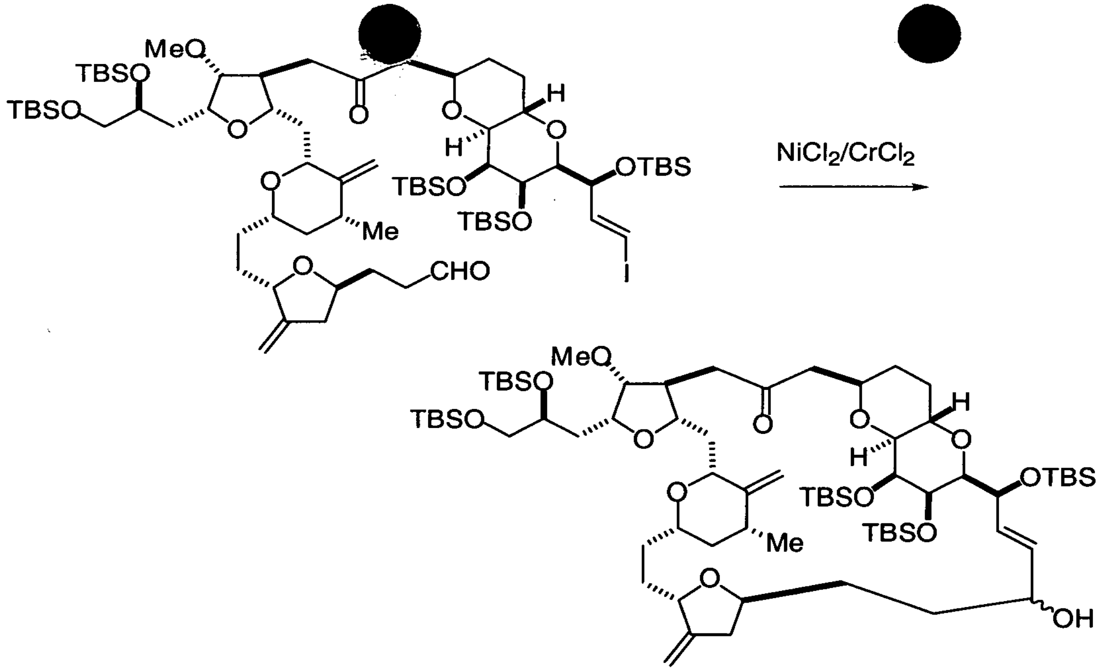


ER804030 **B2304**

Preparation of SmI_2 Solution

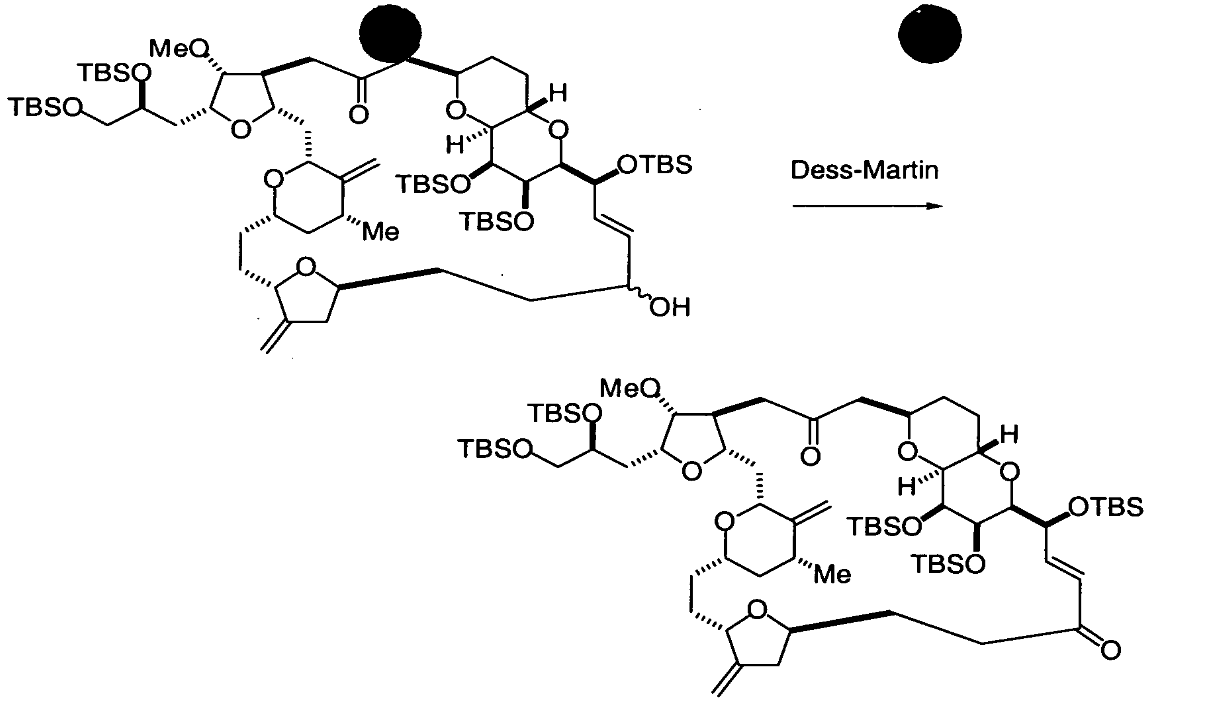
A solution of 1,2-di-iodoethane in 10 mL of THF was added to a suspension of Sm, 0.16 g, in THF, 1 mL. The mixture was stirred for 1 hour.

An aliquot of this solution, 0.03 mL, was added to a solution of the sulfone in THF at -78 ° C. After 5 minutes, additional SmI reagent, 0.05 mL, as added. After a few additional minutes, more reagent, 0.25 mL, was added. The cooling bath was removed and saturated aqueous sodium bicarbonate, 3 mL, was added. The mixture was partitioned between ether and water and the usual work-up gave 9.1 mg, 81%, of an oil.



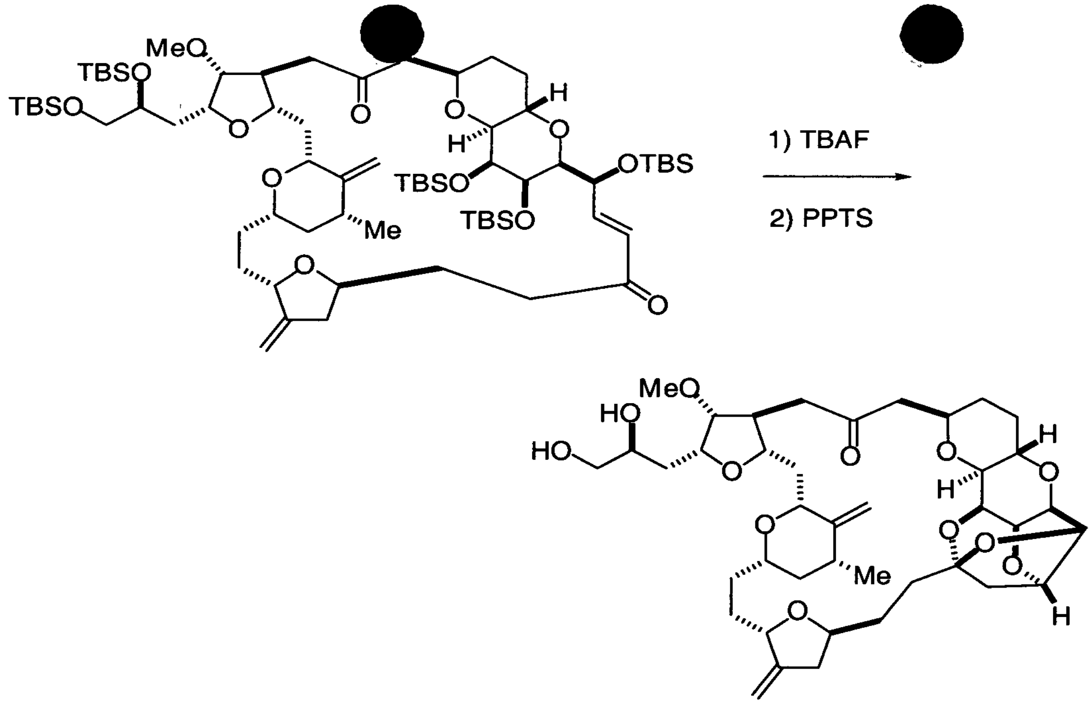
B2304 **B2302, B2303**

B2302 and B2303. In a glove box, $NiCl_2/CrCl_2$ (1% w/w, 1.09 g, 8.86 mmol) was added to a solution of **B2304** (1.01 g, 0.70 mmol) in THF (600 mL) and DMF (150 mL) at rt. After stirring for 2 days the reaction mixture was taken out of the glove box, cooled to 0 °C, quenched with saturated aqueous NH_4Cl (300 mL) and stirred at 0 °C for 20 min. After addition of H_2O (100 mL), the two layers were separated and the aqueous layer was extracted with EtOAc (5×). The combined organic phases were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (15% EtOAc-hexanes) to furnish a mixture of **B2302** and **B2303** (0.84 g, 92%) as a solid foam. Although the isomers could be separated by prep TLC (20% EtOAc-hexanes), they were carried forward as a mixture.



B2302, B2303 **B2301**

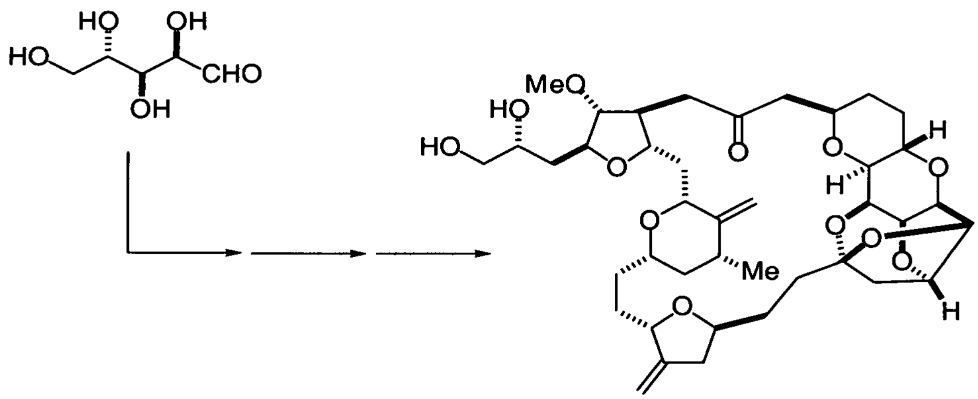
B2301. A mixture of **B2302/B2303** (0.79 g, 0.60 mmol) and Dess-Martin periodinane (0.26 g, 0.60 mmol) in CH_2Cl_2 (30 mL) at rt was stirred for 30 min. Additional Dess-Martin periodinane (0.26 g, 0.60 mmol) was added to the mixture and stirring was continued for additional 1.5 h. The mixture was then diluted with Et_2O (100 mL), stirred for 15 min and filtered through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ (100 mL) and the separated aqueous layer was extracted with Et_2O (3×). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (10% to 15% EtOAc-hexanes) to give **B2301** (0.67 g, 85%) as an oil.



B2301 **B1793**

B1793. TBAF (1 M in THF containing 0.5 M imidazole HCl, 4.60 mL, 4.60 mmol) was added over 2 min to a solution of **B2301** (0.62 g, 0.48 mmol,) in THF (29 mL) at rt and the resulting mixture was stirred for 18 h. After dilution with hexanes (10 mL), the reaction mixture was directly loaded onto a SiO_2 column packed with 50% EtOAc-hexanes and eluted with 50% EtOAc-hexanes (1 L) followed by 10% MeOH/EtOAc to collect a mixture of intermediates. After solvent removal, the residue was dissolved in CH_2Cl_2 (15 mL) and treated with PPTS (645 mg). After stirring for 1 h at rt, additional PPTS (414 mg) was added and the resulting white suspension was stirred for 4.5 h. The reaction mixture was then directly loaded onto a SiO_2 column packed with 70% EtOAc-hexanes and eluted with 70% EtOAc/hexanes (0.5 L), EtOAc (1 L). Elution with 5% to 10% MeOH/EtOAc furnished pure **B1793** (181 mg) and elution with 15% MeOH-EtOAc gave additional semi-pure product, which after purification by preparative TLC (10% MeOH-EtOAc) provided additional pure **B1793** (42 mg). **B1793** (total 223 mg, 64%) was obtained as a white solid. HRMS: calcd for $C_{40}H_{58}O_{12}$ + Na 753.3826. Found: 753.3808.

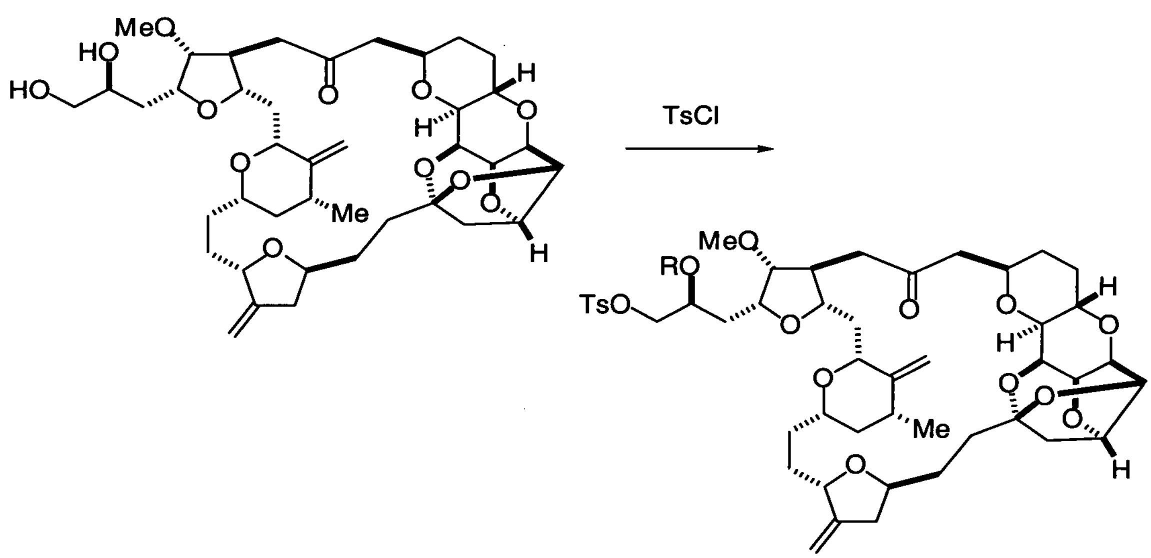
Synthesis of B1794:



Arabinose **B1794**

B1794. Except for stereochemical and protecting group differences (Schemes 3 and 5), arabinose was converted to **B1794** in a manner similar to that described for **B1793** (see schemes 4 and 5). HRMS: calcd for $C_{40}H_{58}O_{12}$ + Na 753.3826. Found: 753.3856.

Synthesis of Representative B1793 Analogs:

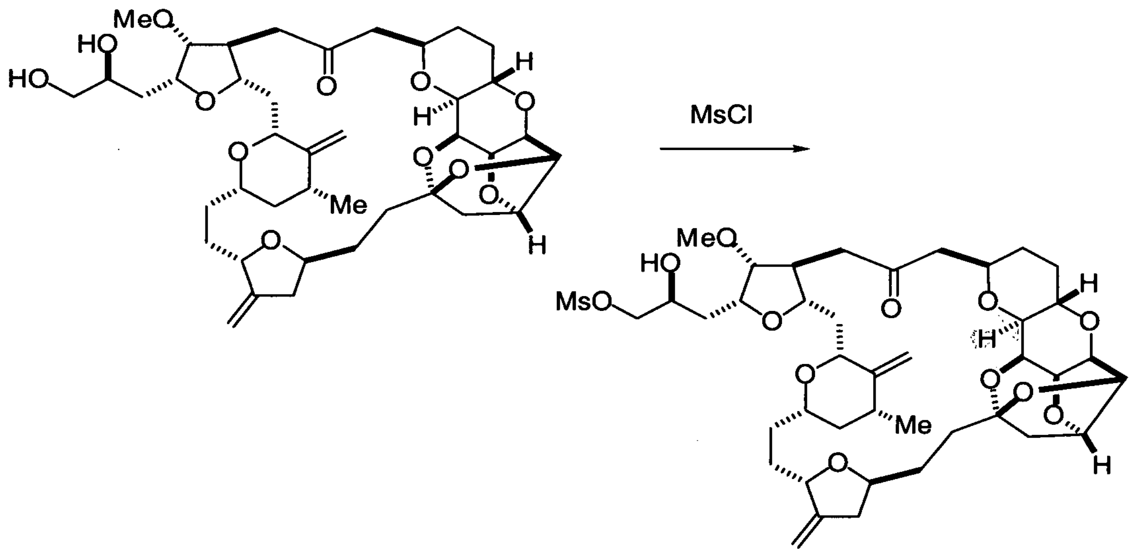


B1793

B1920: R = H
B1921: R = Ts

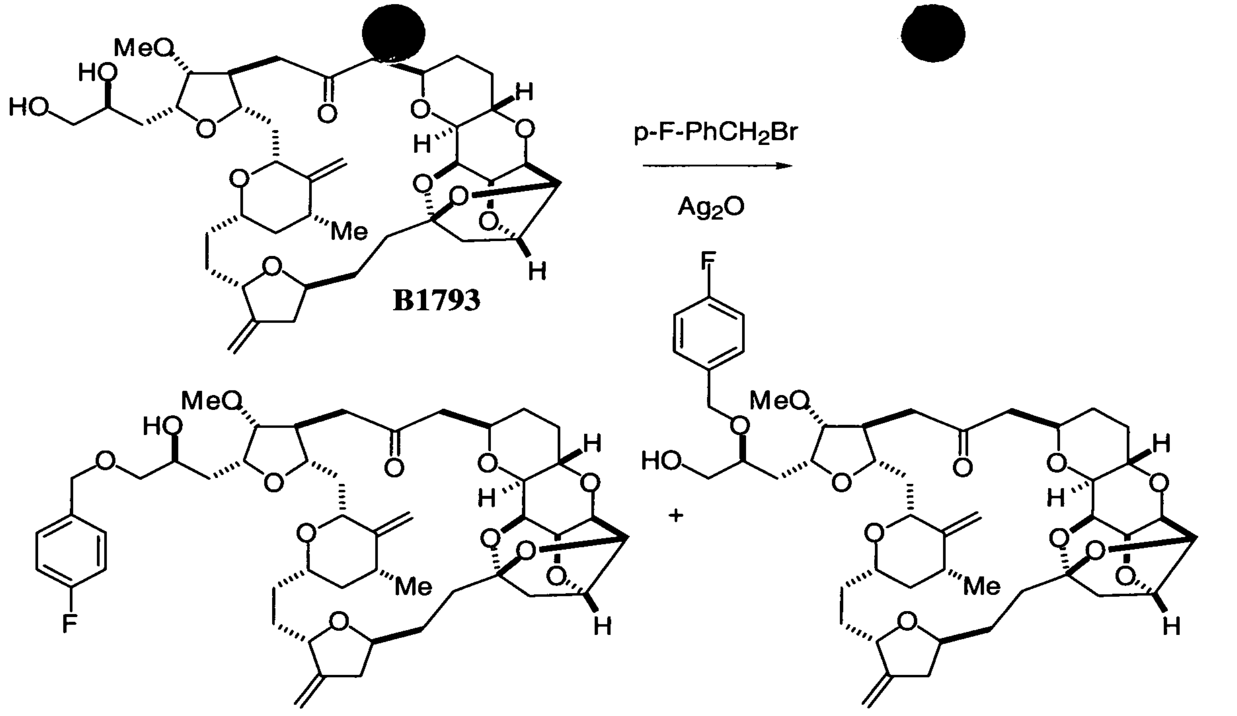
B1920 and B1921 TsCl (9.9 mg, 0.052 mmol) was added to a solution of diol **B1793** (7.6 mg, 0.010 mmol) in CH_2Cl_2 (1 mL) and pyridine (0.1 mL) at rt. After 48 h, the reaction was quenched with a 1:4 mixture of saturated aqueous $NaHCO_3$-brine and extracted with CH_2Cl_2 (4×). The combined extracts were dried over Na_2SO_4 and concentrated. Purification by

preparative TLC (80% EtO[illegible]hexanes) afforded monotosylate **B19[illegible]** ([illegible].0 mg, 67%), and ditosylate **B1921** (1.8 mg, 18%).



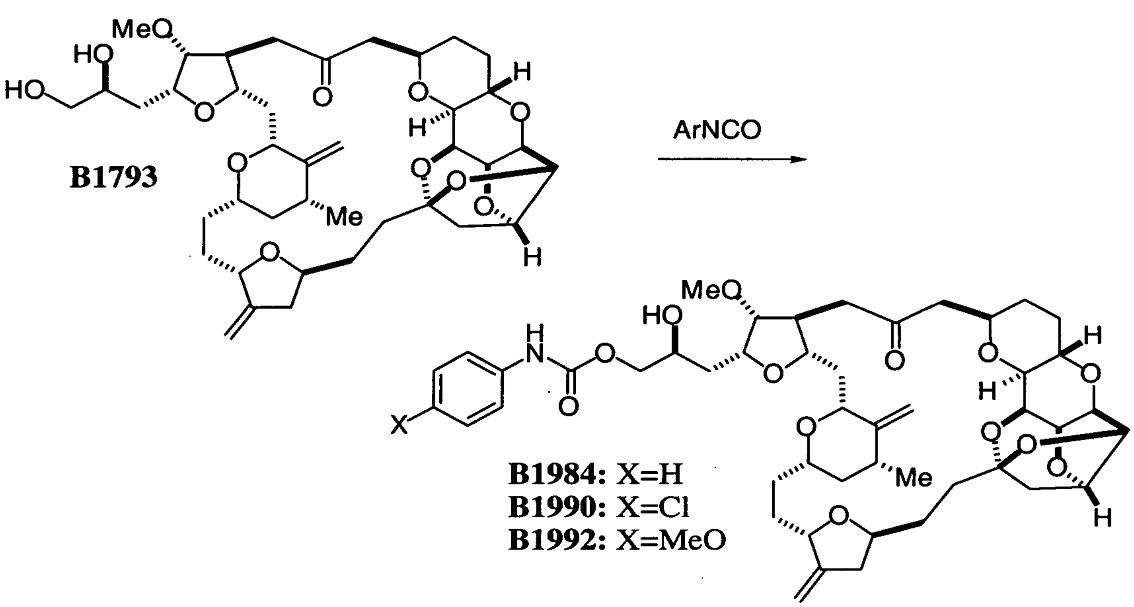
B1793 **B2294**

B2294 MsCl (0.3 M in CH_2Cl_2, 98 μL, 0.030 mmol) was added dropwise over 40 min to a mixture of collidine (7 μL, 0.054 mmol), **B1793** (20.8 mg, 0.028 mmol) and CH_2Cl_2 (1 mL) at 0 °C. After 76 h at 4 °C, the reaction was quenched with a 1:4 mixture of saturated aqueous $NaHCO_3$-brine and extracted with CH_2Cl_2 (4×). The combined extracts were dried over Na_2SO_4 and concentrated. The crude product was dissolved in toluene (3 mL) concentrated and purified by preparative TLC (1.5% MeOH-EtOAc) to afford mesylate **B2294** (21.4 mg, 95%).



B2014 **B2015**

B2014 and B2015 A 0.016 M solution of 4-fluorobenzyl bromide in Et_2O (800 μL, 13 μmol) and Ag_2O (10 mg, 43 μmol) were each added in three portions at 1 h intervals to a rt solution of **B1793** (1.7 mg, 2.3 μmol) in Et_2O (1.2 mL). The mixture was protected from light, stirred for 7 h and then filtered through Celite. Concentration and purification by preparative TLC (EtOAc) afforded primary ether **B2014** (1.1 mg, 56%), and secondary ether **B2015** (0.6 mg, 31%). HRMS (FAB): calcd for $C_{47}H_{63}FO_{12}$ + Na 861.4201. Found: for **B2014** 861.4178, for **B2015** 861.4160.



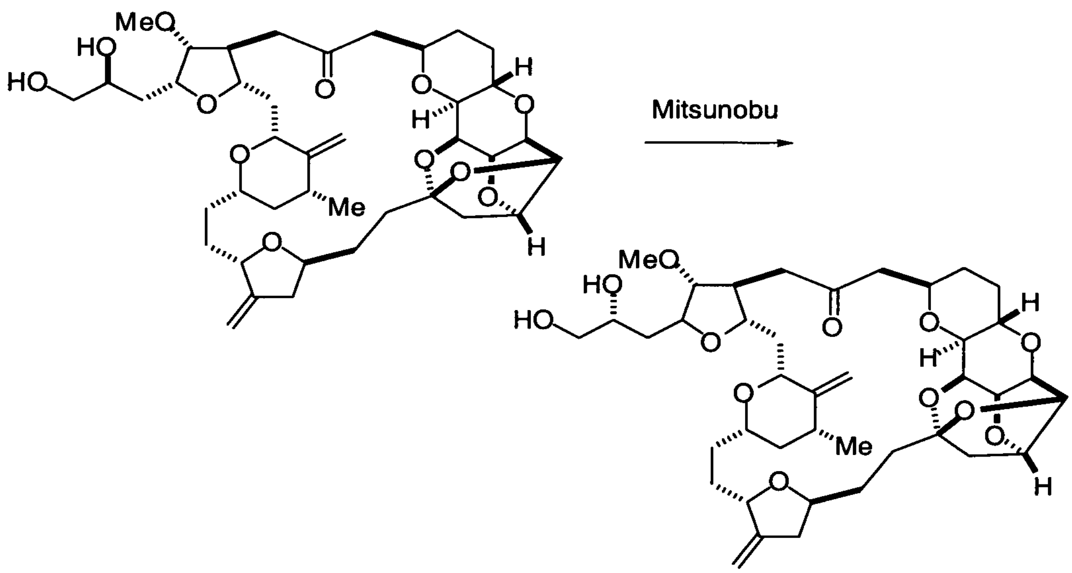
B1984: X=H
B1990: X=Cl
B1992: X=MeO

General. A mixture of **B1793** (1 mg, 1.37 micromol), Et_3N (10 microL, 72 micromol) and ArNCO (2 to 4 equiv.) in CH_2Cl_2 (0.2 mL) was stirred at rt for 4 h to overnight until the reaction was judged to be complete by TLC. The reaction mixture was diluted with saturated $NaHCO_3$ (3 mL), extracted with CH_2Cl_2 (3×) and EtOAc (2×), dried over Na_2SO_4 and purified by preparative TLC (5% MeOH-CH_2Cl_2) to afford the products:

B1984. (1.0 mg, 86%) HRMS (FAB): calcd for $C_{47}H_{63}NO_{13}$ + Na 872.4197. Found: 872.4214.

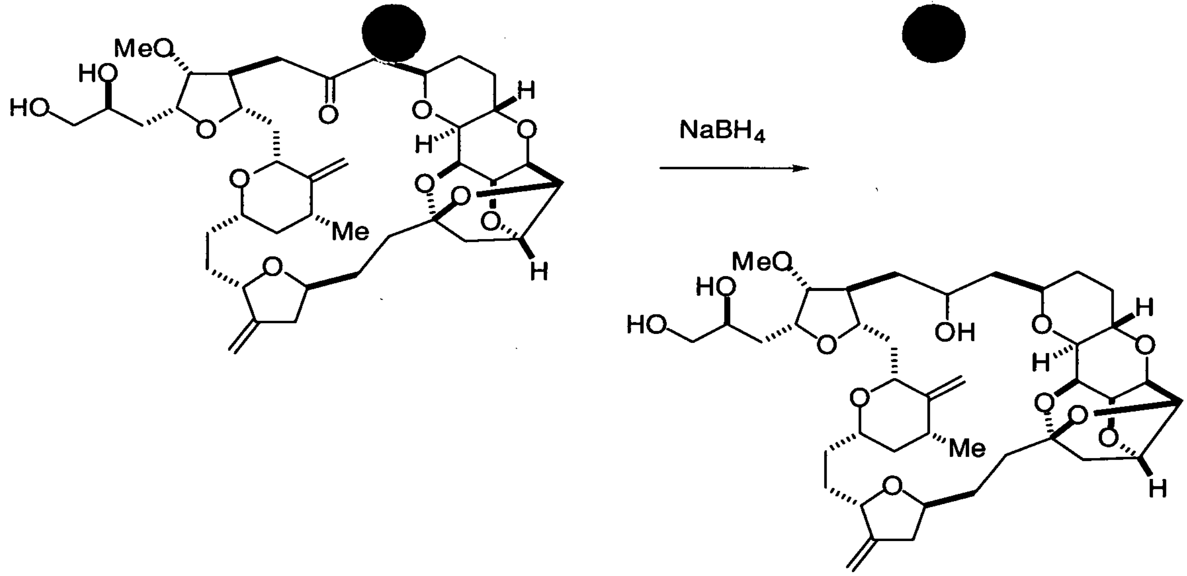
B1990. (1.1 mg, 92%) HRMS (FAB): calcd for $C_{47}H_{62}ClNO_{13}$ + Na 906.3807. Found: 906.3826.

B1992. (1.0 mg, 83%) HRMS (FAB): calcd for $C_{48}H_{65}NO_{14}$ + Na 902.4303. Found: 902.4269.



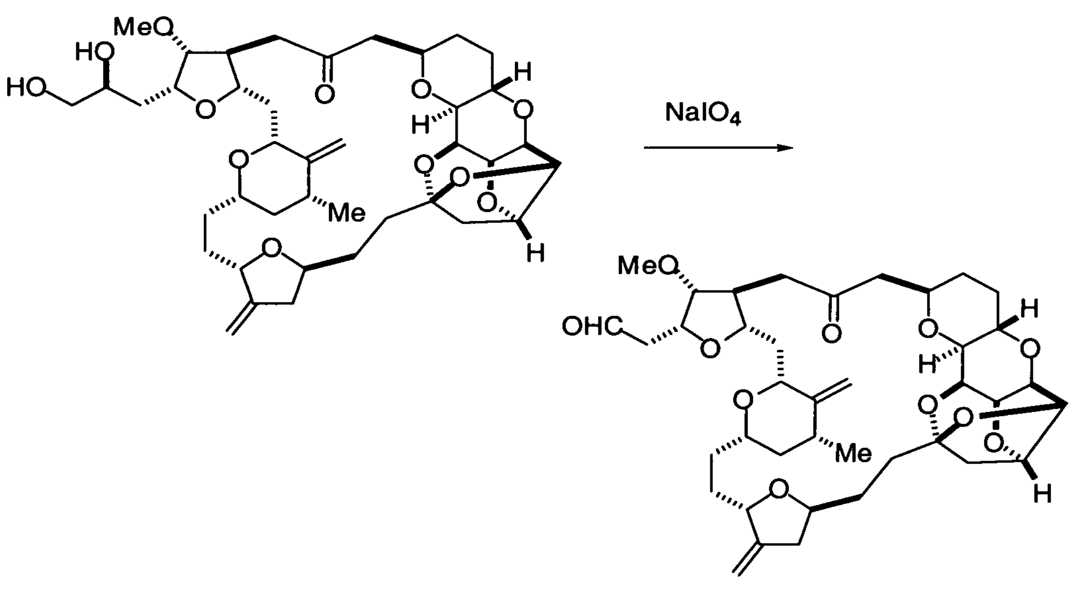
B1793 **B2042**

B2042 DEAD (0.4 M in ether, 50 μL, 19 μmol) was added to a solution of **B1793** (2.0 mg, 2.7 μmol), triphenylphosphine (5 mg, 19 μmol), 4-nitrobenzoic acid (3.2 mg, 19 μmol) and Et_2O (500 μL) at rt. After 22 h, the reaction mixture was loaded directly onto a preparative TLC plate and eluted with 60% EtOAc-hexanes to give the intermediate diester (3.0 mg). This material was taken up in MeOH (300 μL) and treated with K_2CO_3 (approximately 1 mg). After stirring at rt for 30 min, the reaction mixture was diluted with brine and extracted with CH_2Cl_2 (5×). The combined extracts were dried over Na_2SO_4, concentrated and purified by preparative TLC (5% MeOH-EtOAc) to afford **B2042** (1.2 mg, 60% for two steps). HRMS (FAB): calcd for $C_{40}H_{58}O_{12}$ + Na 753.3826. Found: 753.3810.



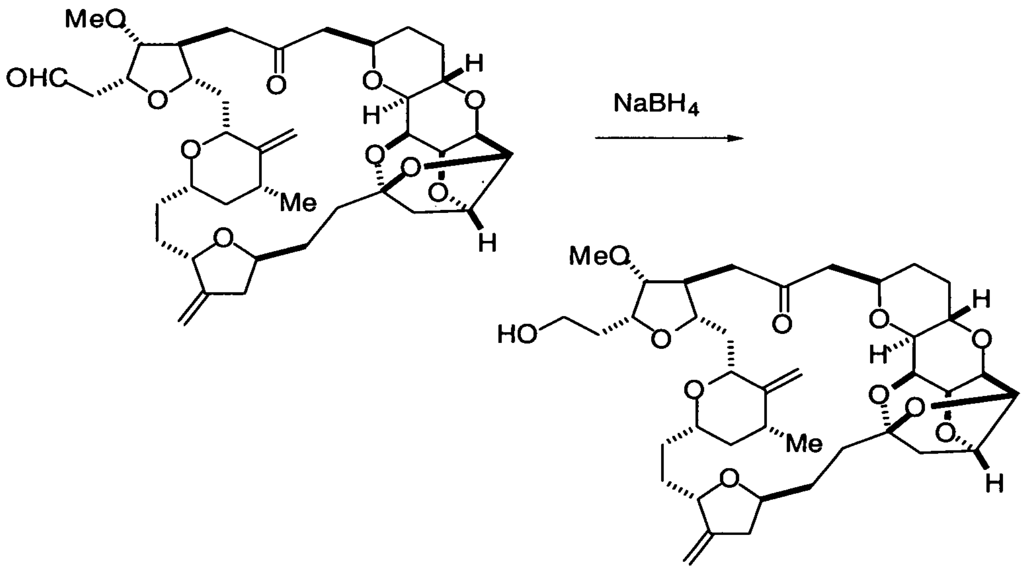
B1793 **B1896, B1897**

B1896 and **B1897.** $NaBH_4$ (3 mg, 0.08 mmol) was added to a solution of **B1793** (2.30 mg, 3.15 μmol) in 1:1 CH_2Cl_2-MeOH (0.2 mL) at rt. Concentration of the reaction mixture and purification by preparative TLC (8% MeOH-EtOAc) provided **B1896** (0.80 mg, 35%) and **B1897** (2:1 mixture, 0.15 mg, 6.5%). HRMS (FAB) for **B1896**: calcd for $C_{40}H_{60}O_{12}$ + Na 755.3983. Found: 753.3969.



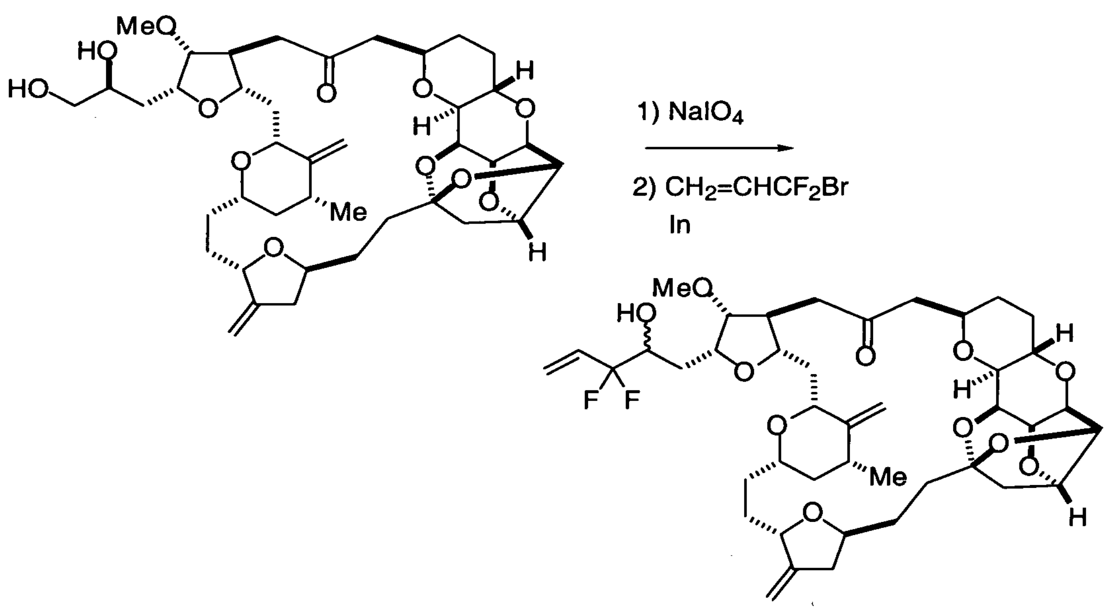
B1793 **B1918**

B1918. A mixture of **B1793** (2.0 mg, 2.74 μmol), $NaIO_4$ (35 mg, 0.16 mmol), MeOH (0.8 mL) and H_2O (0.2 mL) was stirred at rt for 40 min. The reaction mixture was diluted with H_2O (1 mL), extracted with CH_2Cl_2 (6×), dried over Na_2SO_4, concentrated and purified by column chromatography (5% MeOH-CH_2Cl_2) to give **B1918** (1.9 mg, 100%).



B1918 **B2037**

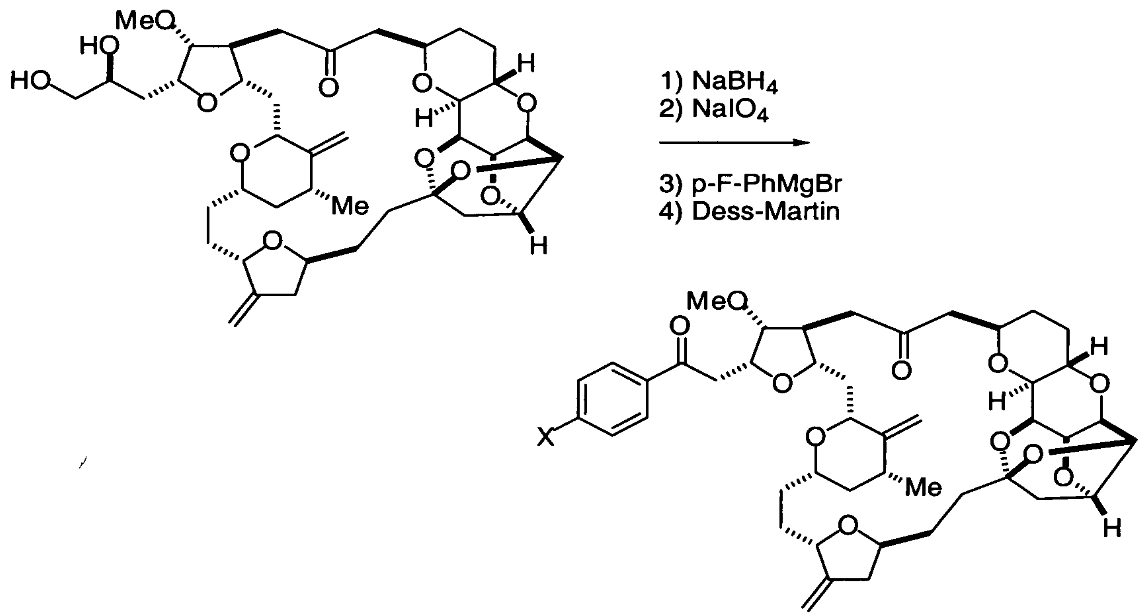
B2037. A 0.034 M solution of $NaBH_4$ (0.1 mL, 3.4 μmol) in EtOH was added portion-wise to a solution of **B1918** (1.9 mg, 2.72 μmol) in MeOH (0.8 mL) and CH_2Cl_2 (0.2 mL) at -78 °C to rt until the reaction was judged to be complete by TLC. The reaction was quenched with saturated aqueous NH_4Cl (2 mL) at -78 °C, warmed to rt, extracted with CH_2Cl_2 (6×), dried over Na_2SO_4 and purified by preparative TLC (5% MeOH- CH_2Cl_2) to afford **B2037** (1.7 mg, 89%). HRMS (FAB): calcd for $C_{39}H_{56}O_{11}$ + Na 723.3720. Found: 723.3749.



B1793 **B2035**

B2035

$NaIO_4$ (35 mg, 0.16 mmol) was added to a solution of **B1793** (1.7 mg, 0.0023 mmol), MeOH (800 μL) and H_2O (200 μL) and after 15 min, the mixture was diluted with H_2O and extracted with CH_2Cl_2 (5×). The organic extracts were dried over Na_2SO_4, concentrated and the intermediate aldehyde was immediately dissolved in DMF (300 μL). 3-Bromo-3,3-difluoropropene (3μL, 0.023 mmol) and indium powder (3 mg, 0.23 mmol) were added and after 24 h additional 3-bromo-3,3-difluoropropene (1 μL, 0.008 mmol) was added. After 18 h, H_2O was added the mixture was extracted with EtOAc (3×). The combined organic extracts were washed successively with H_2O and brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide **B2035** (0.74 mg, 41% for 2 steps) as a mixture of isomers. HRMS (FAB): calcd for $C_{42}H_{58}F_2O_{11}$ + Na 799.3845. Found: 799.3883.



B1793

B2011: X = H
B2008: X = F

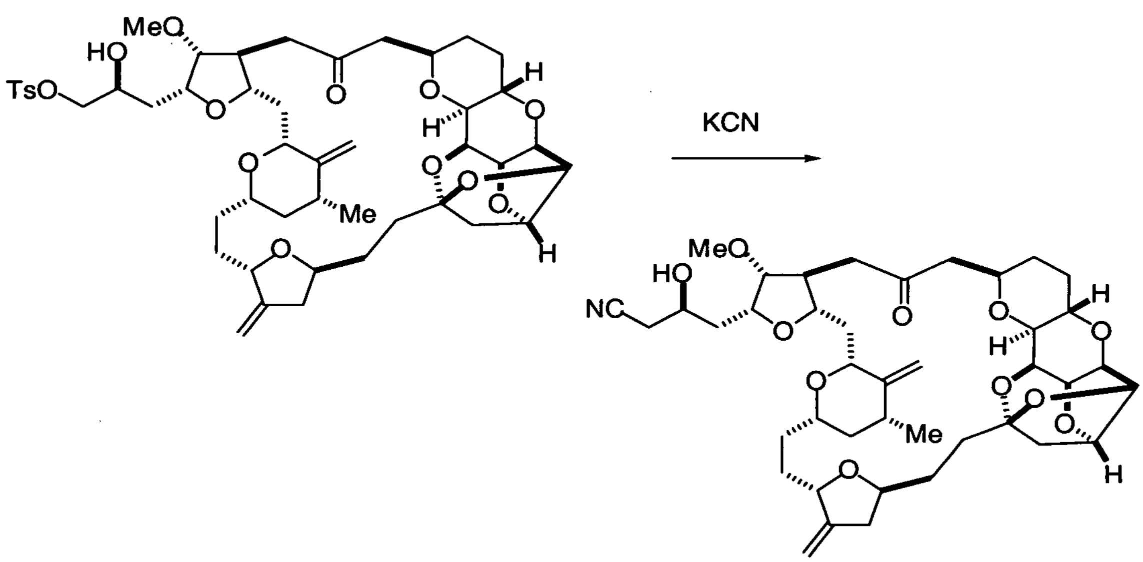
B2008, B2011

$NaBH_4$ (2 mg, 0.05 mmol) was added to a solution of **B1793** (2.2 mg, 0.003 mmol) in 1:1 CH_2Cl_2-MeOH (200 μL) at rt. After 15 min saturated aqueous NH_4Cl and H_2O were added, and the mixture was extracted with CH_2Cl_2 (6×) and EtOAc (2×). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by column chromatography (10% MeOH-EtOAc) to provide an intermediate triol, which was dissolved in MeOH (800 μL) and H_2O (200 μL). $NaIO_4$ (35 mg, 0.16 mmol) was added and after 20 min, the mixture was diluted with H_2O and extracted with CH_2Cl_2 (6×). The organic extracts were dried over

Na_2SO_4, concentrated and the intermediate aldehyde was immediately dissolved in THF (500 μL). 4-Fluorophenylmagnesium bromide (2M in Et_2O, 12 μL, 0.024 mmol) was added and after 20 min the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2 (6×) and the combined organic extracts were dried over Na_2SO_4 and concentrated. Purification by preparative TLC (EtOAc) provided the desired product as a mixture of 4 isomers (1.32 mg, 55% for 3 steps).

Dess-Martin periodinane (~3 mg, 0.007 mmol) was added to a solution of the above product (0.95 mg, 0.0012 mmol) in CH_2Cl_2 (300 μL) and the mixture was stirred at rt for 20 min. Additional Dess-Martin periodinane (~3 mg, 0.007 mmol) and CH_2Cl_2 (300 μL) were added and after another 40 min Et_2O, saturated aqueous $NaHCO_3$ (4 mL) and saturated aqueous $Na_2S_2O_3$ (1 mL) were added. The mixture was extracted with Et_2O (3×) and the combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide **B2008** (0.58 mg, 61%). HRMS (FAB): calcd for $C_{45}H_{57}FO_{11}$ + Na 815.3783. Found: 815.3758.

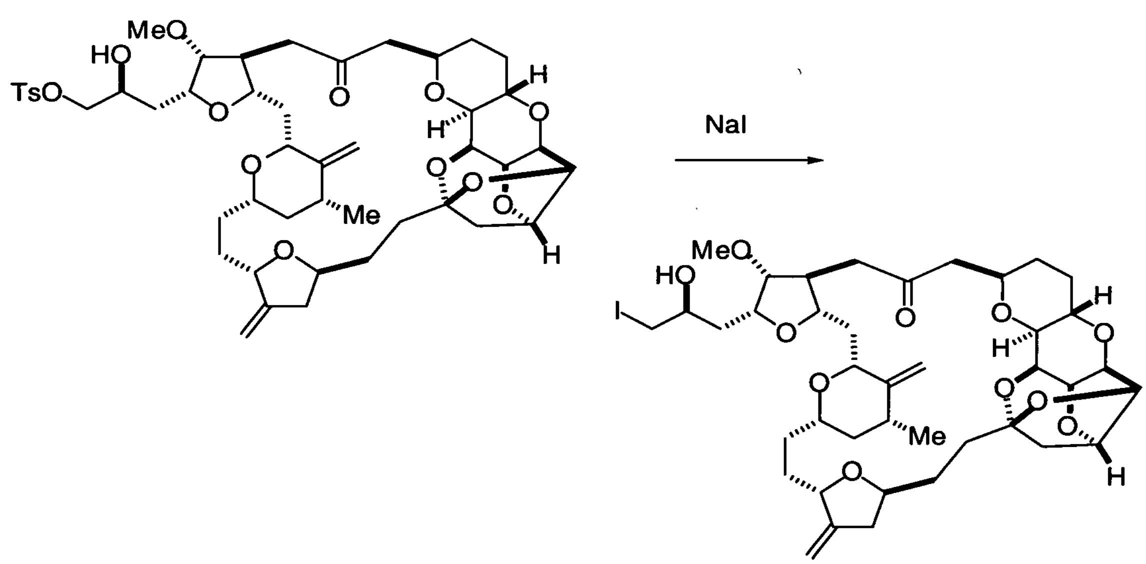
B2011. In an analogous manner, **B1793** (1.9 mg, 0.003 mmol) was converted to **B2011** (0.87 mg, 42% for 4 steps). HRMS (FAB): calcd for $C_{45}H_{58}O_{11}$ + Na 797.3877. Found: 797.3877.



B1920 **B2013**

B2013

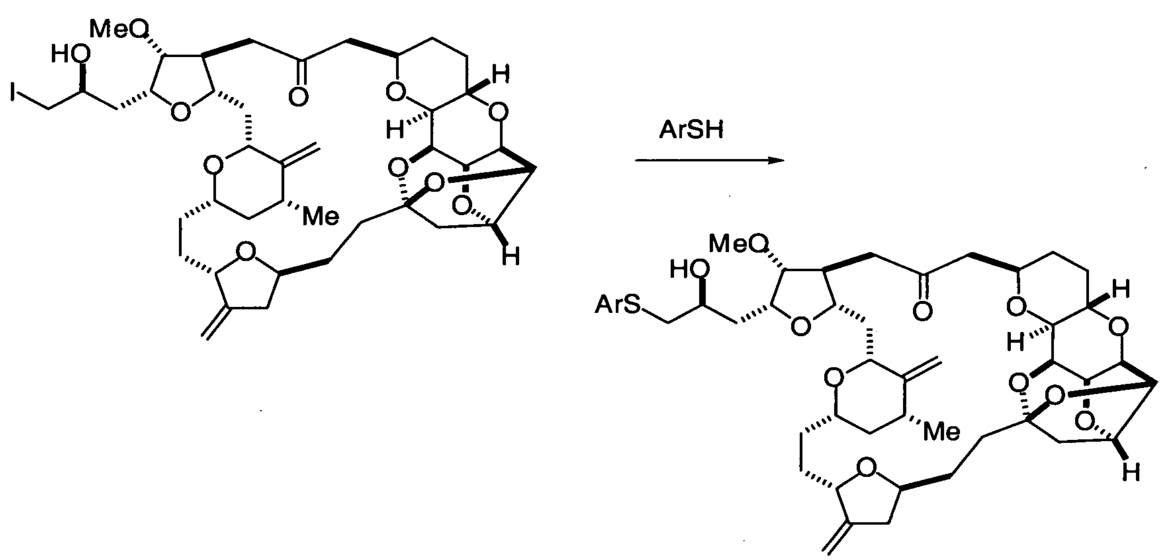
A solution of **B1920** (1.4 mg, 0.0016 mmol), KCN (1 mg, 0.016 mmol) and DMSO (500 μL) was heated at 60 °C for 8 h. After cooling to rt, H_2O was added and the mixture was extracted with EtOAc (3×). The combined organic extracts were washed successively with H_2O and brine, dried over Na_2SO_4 , concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide **B2013** (0.78 mg, 67%). HRMS (FAB): calcd for $C_{41}H_{57}NO_{11}$ + Na 762.3829. Found: 762.3848.



B1920 **X1920**

X1920

A mixture of **B1920** (1.3 mg, 1.47 μmol), NaI (30 mg, excess) and acetone (1 mL) was stirred at 60 °C for 3.5 h. After cooling to rt, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (3 mL), extracted with CH_2Cl_2 (5×) and EtOAc, dried over Na_2SO_4 and purified by column chromatography (50% EtOAc-CH_2Cl_2 to 80% EtOAc-hexanes) to give the iodide **X1920** (1.3 mg, 100%).



X1920

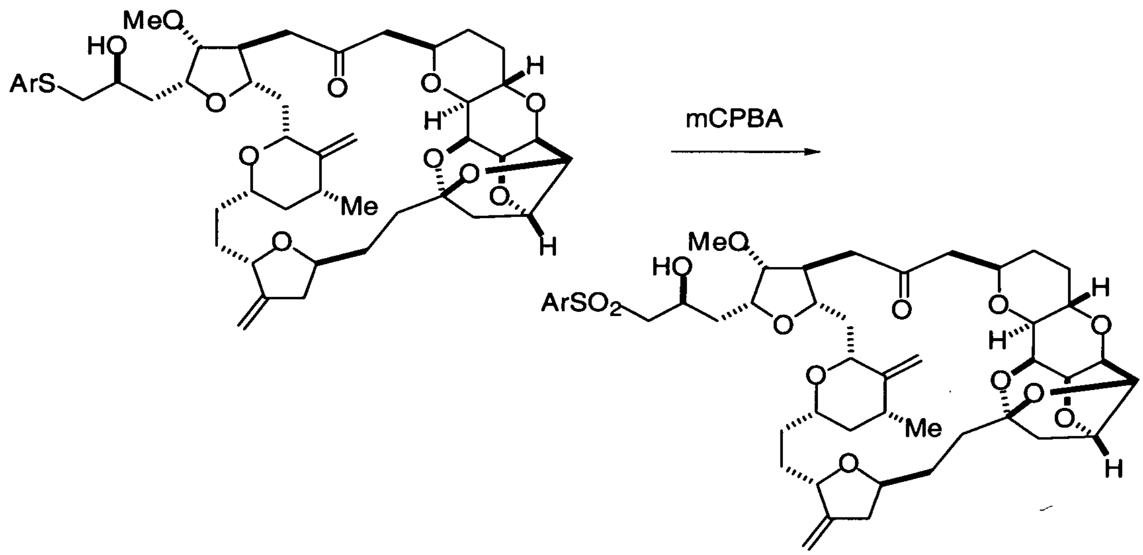
B19[illegible]: Ar = *p*-Cl-Ph
B2010: Ar = *p*-MeO-Ph
B2019: Ar = 2-imidazole

General. A mixture of iodide **X1920** (1.0 equiv.), iPr_2EtN (11 to 22 equiv.), ArSH (9 to 46 equiv.) and DMF (0.3 mL) was stirred at rt until the reaction was judged to be complete by TLC (typically 24 to 48 h). The reaction mixture was diluted with saturated aqueous $NaHCO_3$ (2 mL), extracted with CH_2Cl_2 and EtOAc, dried over Na_2SO_4 and purified by preparative TLC (80% EtOAc-hexanes or 5% MeOH-CH_2Cl_2) to afford the sulfide products:

B1998. (1.3 mg gave 1.1 mg, 85%) HRMS (FAB): calcd for $C_{46}H_{61}ClO_{11}S$ + Na 897.3521. Found: 897.3533.

B2010. (1.1 mg gave 0.7 mg, 59%). HRMS (FAB): calcd for $C_{47}H_{64}O_{12}S$ + Na 875.4016. Found: 875.4057.

B2019. (1.1 mg gave 0.7 mg, 61%) MS (FAB): M + Na



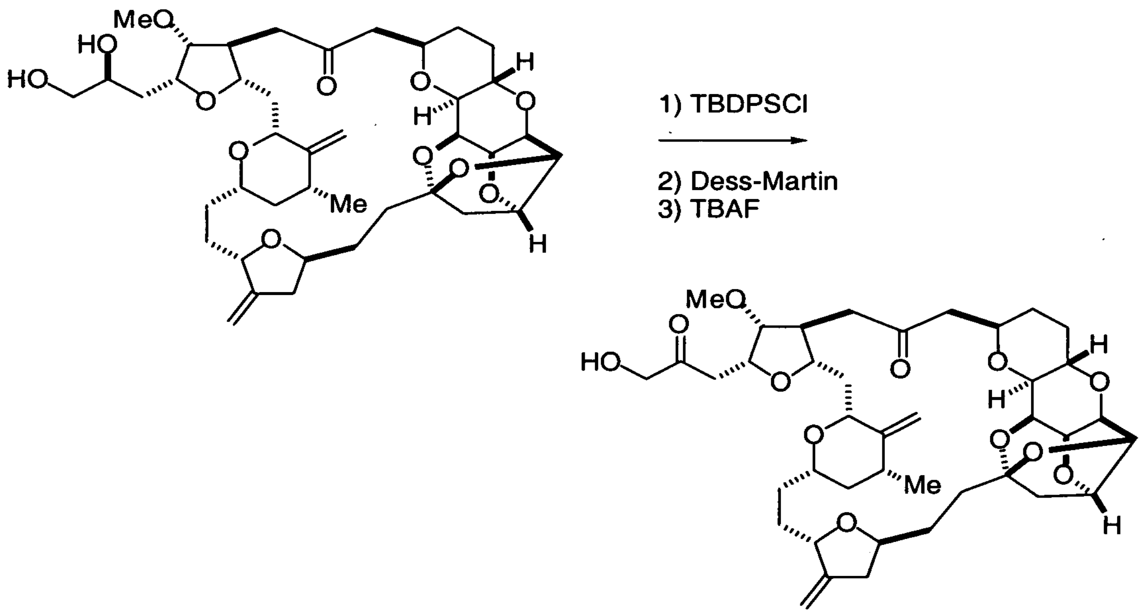
B1998: Ar = *p*-Cl-Ph
B2010: Ar = *p*-MeO-Ph

B2016: Ar = *p*-Cl-Ph
B2030: Ar = *p*-MeO-Ph

General. A 0.01 M solution of mCPBA (1.2 equiv.) in CH_2Cl_2 was added to a solution of a sulfide (1.0 equiv.) in CH_2Cl_2 (0.5 mL) at 0 °C for 30 min. The reaction mixture was diluted with saturated $NaHCO_3$ (2 mL), extracted with CH_2Cl_2 and EtOAc, dried over Na_2SO_4 and purified by preparative TLC (80% EtOAc-hexanes or EtOAc) to afford the products:

B2016. (0.9 mg gave 0.7 mg, 74%)

B2030. (1.0 mg gave 0.6 mg, 61%) HRMS (FAB): calcd for $C_{47}H_{64}O_{14}S$ + Na 907.3914. Found: 907.3950.

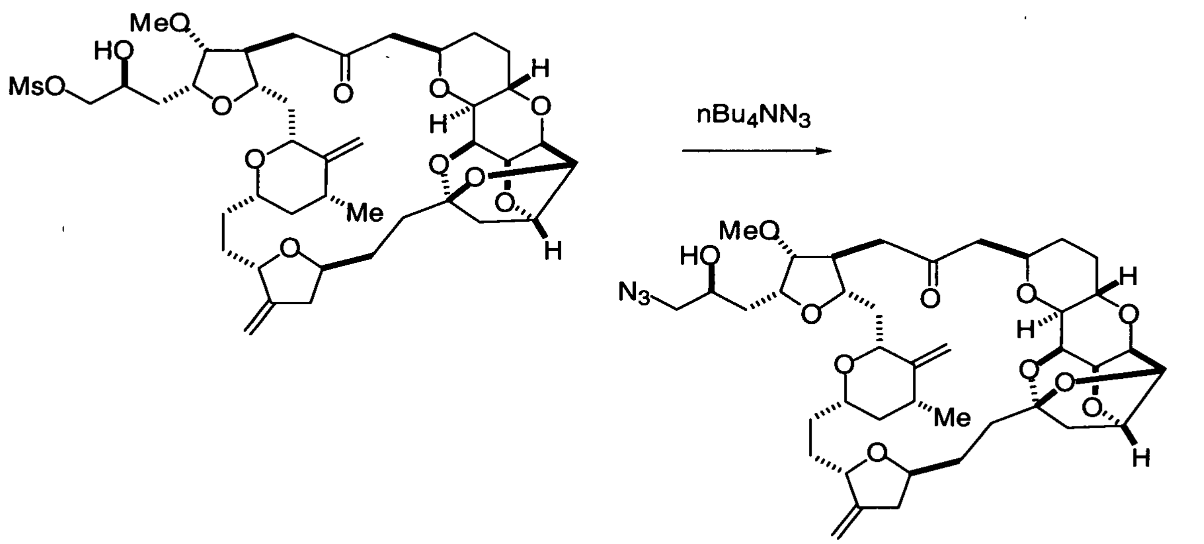


B1793 **B1934**

B1934. TBDPSCl (3.0 μL, 12 μmol) was added to a solution of **B1793** (1.3 mg, 1.78 μmol), imidazole (10 mg, 166 μmol) and DMF (0.10 mL) at rt. After stirring for 1 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (2 mL), extracted with CH_2Cl_2 (3×) and EtOAc (2×), dried over Na_2SO_4 and purified by preparative TLC (5% MeOH-CH_2Cl_2) to give the intermediate silyl ether (1.3 mg, 77%).

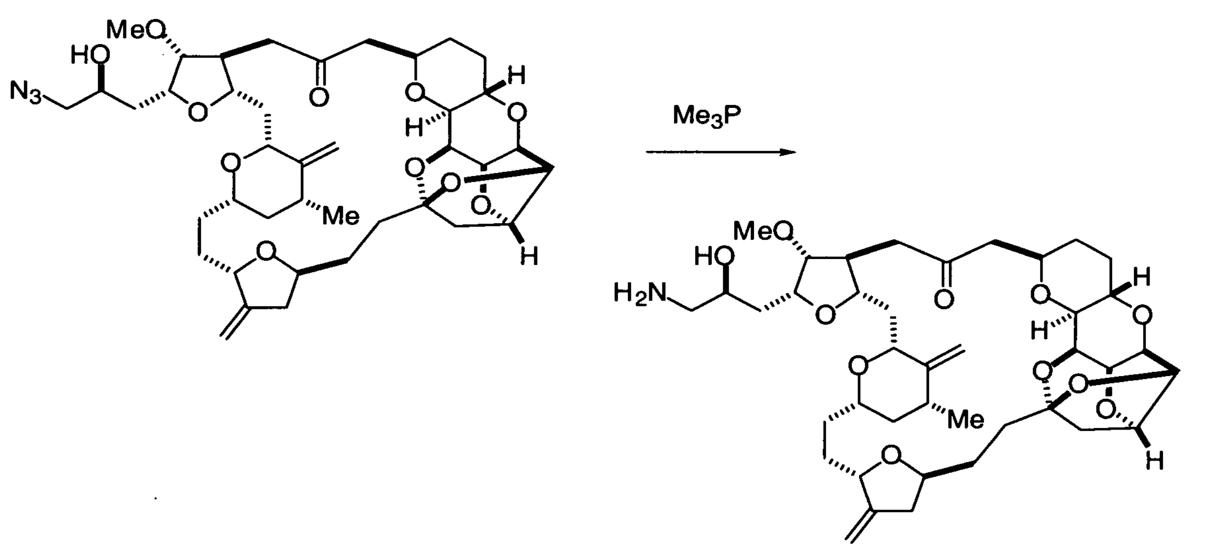
This material was dissolved in CH_2Cl_2 (0.5 mL) and treated with Dess-Martin periodinane (10 mg, 24 μmol) for 1.5 h at rt, diluted with Et_2O and filtered through Celite. The filtrate was concentrated and purified by preparative TLC (50% EtOAc-hexanes) to afford the diketone intermediate (1.0 mg, 77%), which was dissolved in THF (0.5 mL) and treated with 0.02 M TBAF containing 0.01 M imidazole hydrochloride (THF solution, 75 μL, 1.5 μmol) at rt for 15 min. The reaction mixture was eluted through a SiO_2 column (50% EtOAc-hexanes to 5% MeOH-CH_2Cl_2) and the desired product was further purified by preparative TLC (5% MeOH-CH_2Cl_2) to afford **B1934** (0.75 mg, 100%). HRMS (FAB): calcd for $C_{40}H_{56}O_{12}$ + Na 751.3669. Found: 751.3690.

Synthesis of B1939:



B2294 **B1922**

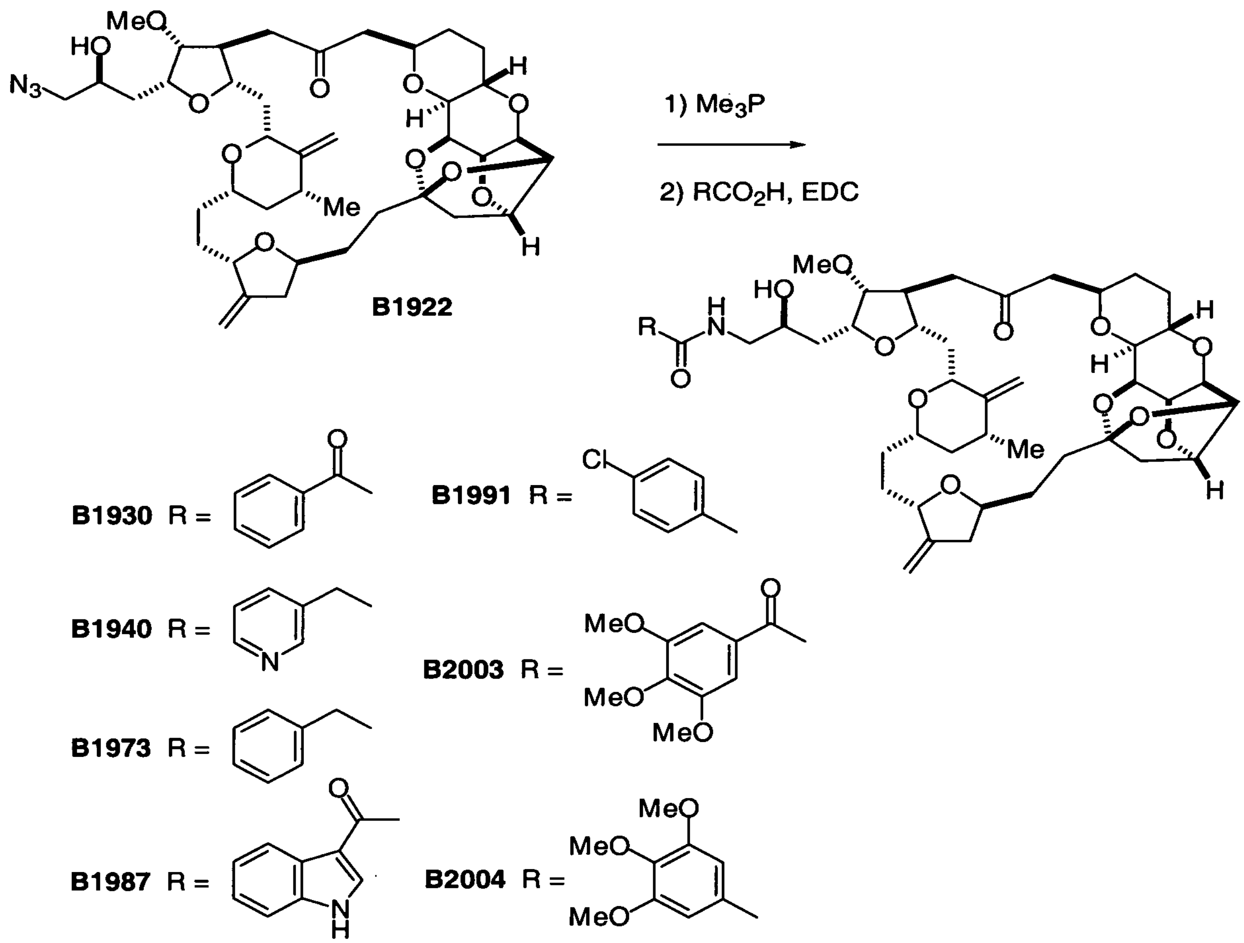
B1922 Tetra-n-butylammonium azide (0.2 M in DMF, 0.5 mL, 0.10 mmol) was added to a solution of mesylate **B2294** (21.4 mg, 0.026 mmol) in DMF (2 mL) at rt. After stirring at 83 °C for 3.5 h, the reaction mixture was cooled to rt, diluted with toluene, concentrated and purified by preparative TLC (80% ethyl acetate-hexanes) to furnish **B1922** (18 mg, 92%).



B1922 **B1939**

B1939 Me_3P (1 M in THF) and H_2O (0.8 mL) were sequentially added to a solution of azide **B1922** (24.6 mg, 0.032 mmol) in THF (3.2 mL) at rt. The mixture was stirred for 22 h, diluted with toluene, concentrated and purified by flash chromatography [step gradient, 10% MeOH-EtOAc followed by MeOH-EtOAc-30% aqueous NH_4OH (9:86:5)] to provide the desired primary amine (23.3 mg), which by ^1H-NMR contained ~1% trimethylphosphine oxide. Lyophilization from benzene and standing under high vacuum for 2 d furnished **B1939** (20.3 mg, 87%).

Synthesis of Representative B1939 Analogs:
B1930, B1940, B1973, B1987, B1988, B1991, B2003, B2004



B1930 Me_3P (1 M in THF, 13 μL, 0.013 mmol) was added to a solution of **B1922** (1.6 mg, 2.1 μmol), THF (400 μL) and H_2O (100 μL) at rt. The mixture was stirred for 22 h, diluted with toluene, concentrated, and azeotropically dried with toluene (2x) to give the crude amine which was used directly in the next step.

EDC (0.06 M in CH_2Cl_2, 100 μL, 11 μmol) was added to a solution of the crude amine, benzoylformic acid (0.8 mg, 5.3 μmol) and CH_2Cl_2 (200 μL) at rt. After 30 min, the reaction

was quenched with a 1:4 mixture of saturated aqueous $NaHCO_3$-brine and extracted with CH_2Cl_2 (5×). The combined extracts were dried over Na_2SO_4 and concentrated and purified by preparative TLC (EtOAc) to afford **B1930** (1.5 mg, 83% for two steps). HRMS (FAB): calcd for $C_{48}H_{63}NO_{13}$ + Na 884.4197. Found: 884.4166.

B1940 Using the procedure described above for **B1930**, **B1922** was reduced, coupled with 3-pyridylacetic acid hydrochloride and purified by preparative TLC [(MeOH-EtOAc-30% aqueous NH_4OH (9:86:5)] to afford **B1940** (0.8 mg, 67 % for two steps). HRMS (FAB): calcd for $C_{47}H_{64}N_2O_{12}$ + Na 871.4357. Found: 871.4362.

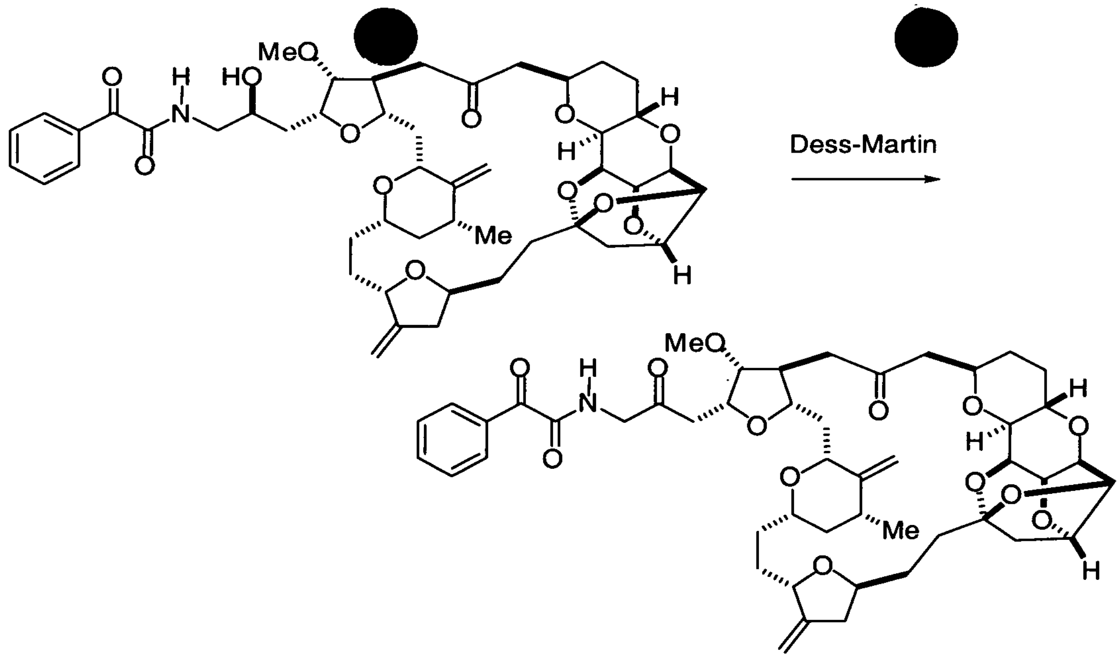
B1973 Using the procedure described above, **B1922** (0.9 mg, 1.2 μmol) was reduced, coupled with phenylacetic acid and purified by preparative TLC (5% MeOH-EtOAc) to afford **B1973** (0.44 mg, 44 % for two steps). HRMS (FAB): calcd for $C_{48}H_{65}NO_{12}$ + Na 870.4404. Found: 870.4447.

B1987 Using the procedure described above, **B1922** (0.9 mg, 1.2 μmol) was reduced, coupled with 3-indoleglyoxylic acid and purified by preparative TLC (3% MeOH-EtOAc) to afford **B1987** (0.8 mg, 75 % for two steps). HRMS (FAB): calcd for $C_{50}H_{64}N_2O_{12}$ + Na 923.4306. Found: 923.4338.

B1991 Using the procedure described above, **B1922** (1.0 mg, 1.3 μmol) was reduced, coupled with 4-chlorobenzoic acid and purified by preparative TLC (3% MeOH-EtOAc) to afford **B1991** (0.8 mg, 70 % for two steps). HRMS (FAB): calcd for $C_{47}H_{62}ClNO_{12}$ + Na 890.3858. Found: 890.3843.

B2003 Using the procedure described above, **B1922** (1.0 mg, 1.3 μmol) was reduced, coupled with 3,4,5-trimethoxybenzoylformic acid and purified by preparative TLC (EtOAc) to afford **B2003** (0.7 mg, 56 % for two steps). HRMS (FAB): calcd for $C_{51}H_{69}NO_{16}$ + Na 974.4514. Found: 974.4525.

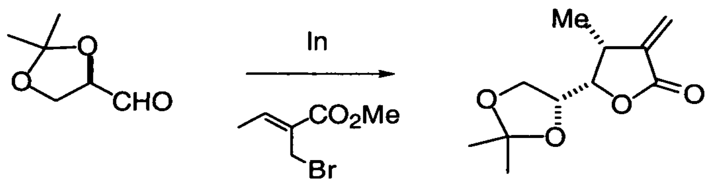
B2004 Using the procedure described above, **B1922** (1.0 mg, 1.3 μmol) was reduced, coupled with 3,4,5-trimethoxybenzoic acid and purified by preparative TLC (5% MeOH-EtOAc) to afford **B2004** (0.7 mg, 58 % for two steps). HRMS (FAB): calcd for $C_{49}H_{65}NO_{13}$ + Na 946.4565. Found: 946.4599.



B1930 **B1988**

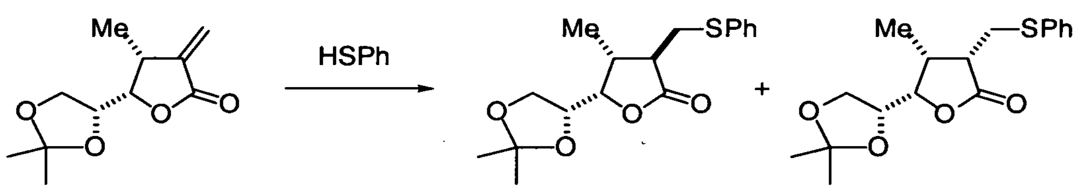
B1988 Dess-Martin periodinane (1 mg, 2.3 μmol) was added to a solution of **B1930** (0.80 mg, 0.93 μmol) in CH_2Cl_2 (500 μL) at rt. After 1 h, the reaction was diluted with Et_2O and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous $NaHCO_3$-$Na_2S_2O_3$ and brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to afford **B1988** (0.45 mg, 56%). HRMS (FAB): calcd for $C_{48}H_{61}NO_{13}$ + Na 882.4041. Found: 884.4012.

Synthesis of B2090:



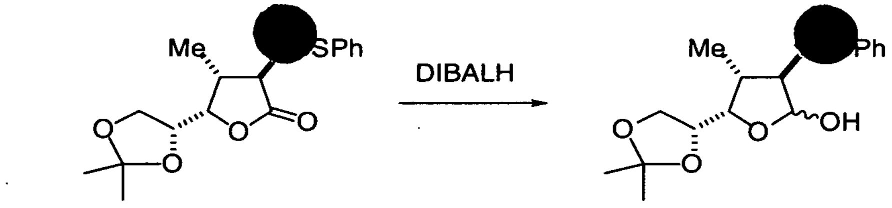
101 **102** **103**

Compound 103. Indium powder (1.35 g, 11.8 mmol) was added to a solution of **102** (3.38 g, 17.6 mmol) in DMF (20 mL) at rt. After stirring for 30 min, the reaction mixture was cooled to 0 °C. Neat aldehyde **101** (3.72 g, 28.6 mmol) was then added and the mixture was stirred overnight while allowing the temperature to warm to rt. The reaction mixture was recooled to 0 °C and then quenched carefully with saturated aqueous NH_4Cl (100 mL). After stirring for 30 min, the resulting mixture was extracted with Et_2O (3×), dried over Na_2SO_4, concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to give pure crystalline **103** (2.20 g, 59%).



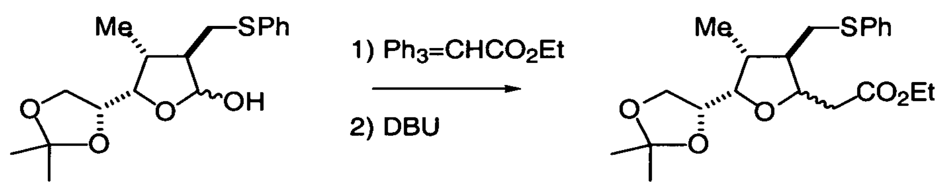
103 **104** **105**

Compound 104. Et_3N (72μL, 0.51 μmol) was added to a solution of **103** (1.09 g, 5.13 mmol) and thiophenol (0.63 mL, 7.16 mmol) in CH_2Cl_2 and the resulting mixture was stirred at 0 °C for 1 h. Filtration through SiO_2 gave a mixture of **104** and **105**, which after MPLC (15% to 20% EtOAc-hexanes) afforded **104** (0.53 g, 32%) and **105** (0.92 g, 56%).



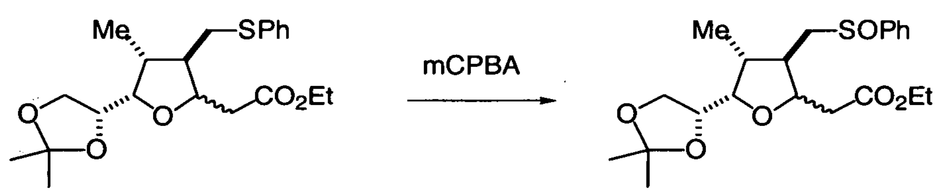
104 **106**

Compound 106. DIBALH (1 M in toluene, 3.28 mL, 3.28 mmol) was added to a solution of **104** (0.53 g, 1.64 mmol) in toluene (10 mL) at -78 °C and the mixture was stirred at -78°C for 10 min. The reaction was quenched by careful addition of MeOH (0.40 mL, 9.84 mmol) and H_2O (0.17 mL, 9.84 mmol), warmed to rt and stirred for 20 min. The white suspension was filtered through a mixture of Celite and SiO_2 with 1:1 CH_2Cl_2-Et_2O and concentrated to give **106** (0.53 g, 100%) as an oil.



106 **107**

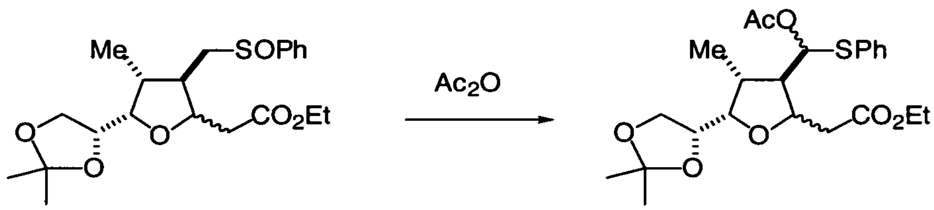
Compound 107. A mixture of **106** (0.53 g, 1.64 mmol) and ethyl (triphenylphosphoranylidene)acetate (1.15 g, 3.29 mmol) in toluene (10 mL) was heated to 80 °C for 15 h. The mixture was cooled to rt and DBU (25 μL, 0.16 mmol) was introduced. The mixture was heated to 80 °C for 1.5 h, cooled to rt, concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to give **107** (0.54 g, 83%) as an oil (3:1 ratio of α:β isomers).



107 **108**

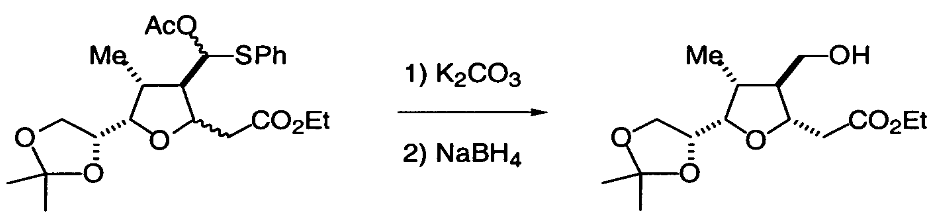
Compound 108. A solution of mCPBA (~55%, 450 mg in 4.5 mL CH_2Cl_2, 1.44 mmol) was added to a solution of **107** (0.54 g, 1.36 mmol) in CH_2Cl_2 (10 mL) at -78 °C. The

reaction mixture was diluted with saturated aqueous $NaHCO_3$ (50 mL), H_2O (10 mL), and Et_2O (60 mL) and then warmed to rt. The separated aqueous layer was extracted with EtOAc (4×) and the combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (50% EtOAc-hexanes) to give **108** (0.51 g, 92%) as an oil.



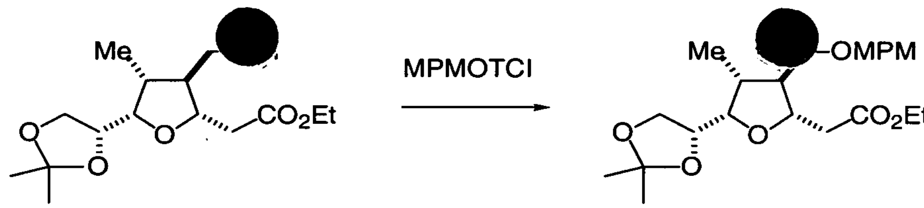
108 **109**

Compound 109. A mixture of **108** (0.51 g, 1.24 mmol) and NaOAc (1.00 g, 12.4 mmol) in Ac_2O (10 mL) was stirred at 140 °C for 12 h, cooled to rt and then concentrated. The residue was partitioned between saturated aqueous $NaHCO_3$ (20 mL) and Et_2O (30 mL), and stirred vigorously at rt for 30 min. The separated aqueous layer was extracted with Et_2O (2×), and the combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (5% to 15% EtOAc-hexanes) to give **109** (0.41 g, 73%) as an oil.



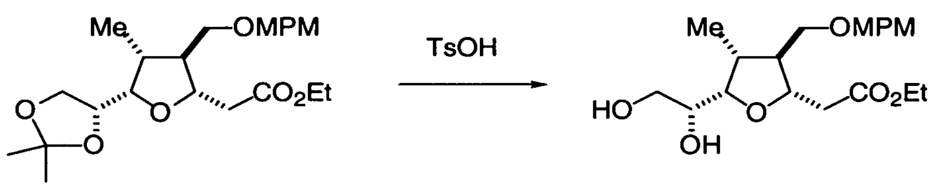
109 **110**

Compound 110. A mixture of **109** (0.41 g, 0.91 mmol) and K_2CO_3 (44.3 mg, 0.32 mmol) in EtOH (5 mL) was heated to 60-70 °C for 1 d. After cooling to rt, the reaction mixture was concentrated and eluted through a SiO_2 column (10% to 20% EtOAc-hexanes) to give the partially purified aldehyde intermediate. This material was dissolved in EtOH (2.5 mL), treated with $NaBH_4$ (50 mg, 1.32 mmol) and stirred at rt for 30 min. The mixture was concentrated and purified by column chromatography (40% EtOAc-hexanes) to give **110** (181 mg, 66%).



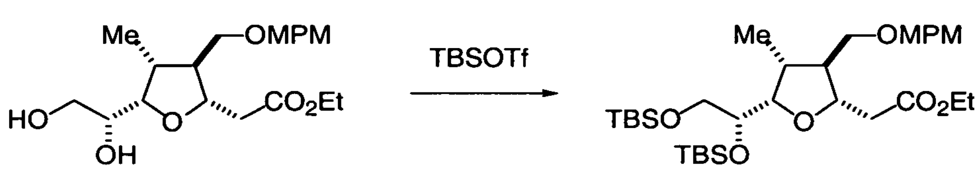
110 **111**

Compound 111. BF_3•OEt_2 (0.05 M in CH_2Cl_2, 175 μL, 8.75 μmol) was added to a solution of **110** (181 mg, 0.60 mmol) and *p*-methoxybenzyl 2,2,2-trichloroacetimidate (0.50 mL, 1.80 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The resulting mixture was stirred for 1.5 h at 0 °C and for 2 h at rt until the reaction was complete. The mixture was quenched with saturated aqueous $NaHCO_3$ (25 mL) and extracted with Et_2O (5×). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (CH_2Cl_2 and then 20% EtOAc-hexanes) to give semi-pure **111** (0.37 g, >100%) as an oil.



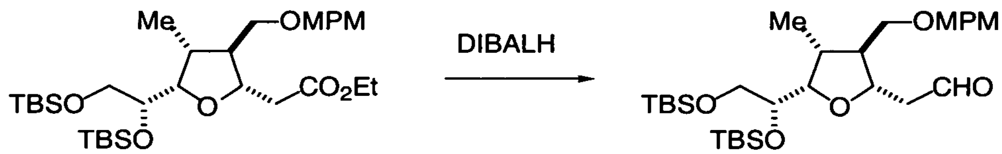
111 **112**

Compound 112. A mixture of **111** (0.37 g, max.= 0.60 mmol) and TsOH•H_2O (36 mg) in EtOH (5 mL) was stirred initially at rt overnight and then at 60 °C for 1 h. Additional TsOH•H_2O (31 mg) was added at rt and the reaction mixture was stirred for 1 h at rt. The mixture was then concentrated, quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc (5×). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (20% to 50% EtOAc-hexanes and then 5% MeOH-CH_2Cl_2) to give **112** (121 mg, 53%) as an oil along with recovered **111** (49 mg, 21%).



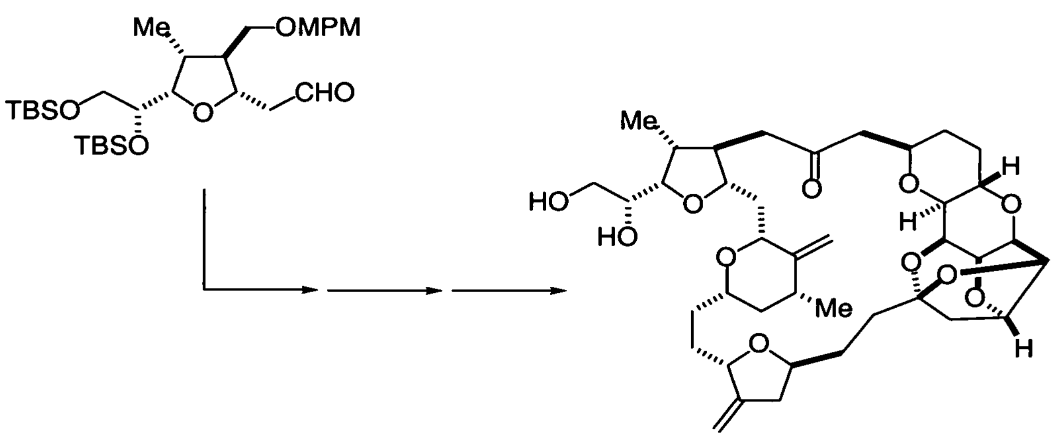
112 **113**

Compound 113. TBSOTf (250 μL, 1.09 mmol) was added to a solution of **112** (121 mg, 0.32 mmol) and Et_3N (176 μL, 1.26 mmol) in CH_2Cl_2 at 0 °C and the resulting mixture was stirred for 25 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (15 mL) and the separated aqueous layer was extracted with ether (3×). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (5% to 10% EtOAc/hexanes) to give **113** (165 mg, 85%) as an oil.



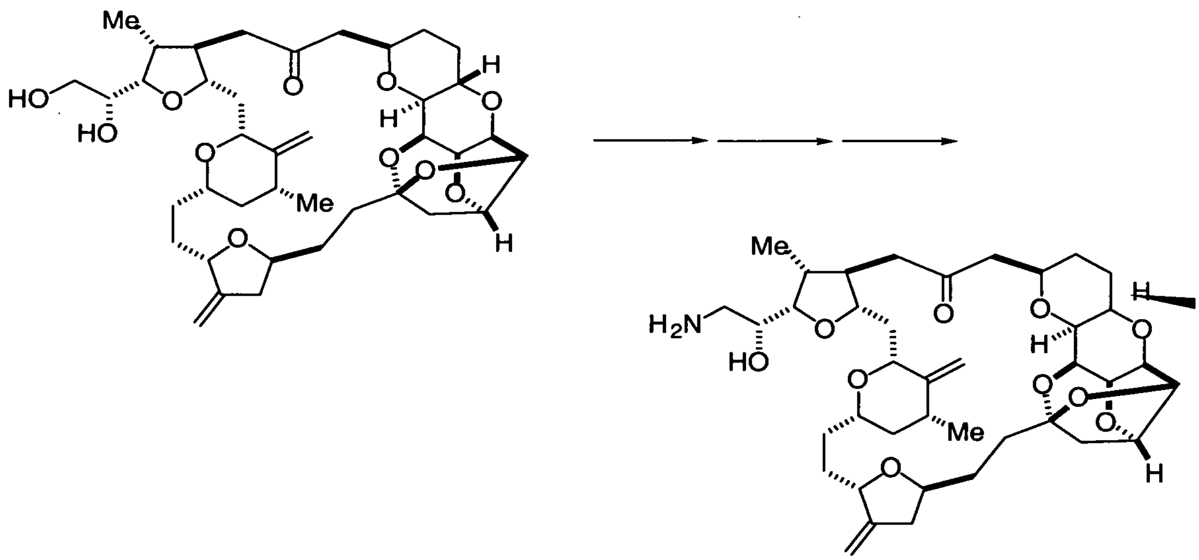
113 **114**

Compound 114. DIBALH (1 M in toluene, 0.54 mL, 0.54 mmol) was added to a solution of **113** (165 mg, 0.27 mmol) in toluene (5 mL) at -78 °C and the resulting mixture was stirred at -78 °C for 10 min. The reaction was quenched by careful addition of MeOH (65 μL, 0.81 mmol) and H_2O (29 μL, 0.81 mmol), warmed to rt and stirred for 25 min. The white suspension was filtered through Celite with 1:1 CH_2Cl_2-Et_2O. Concentration and purification by column chromatography (10% to 20% EtOAc-hexanes) gave **114** (153 mg, 100%) as an oil.



114 **B2090**

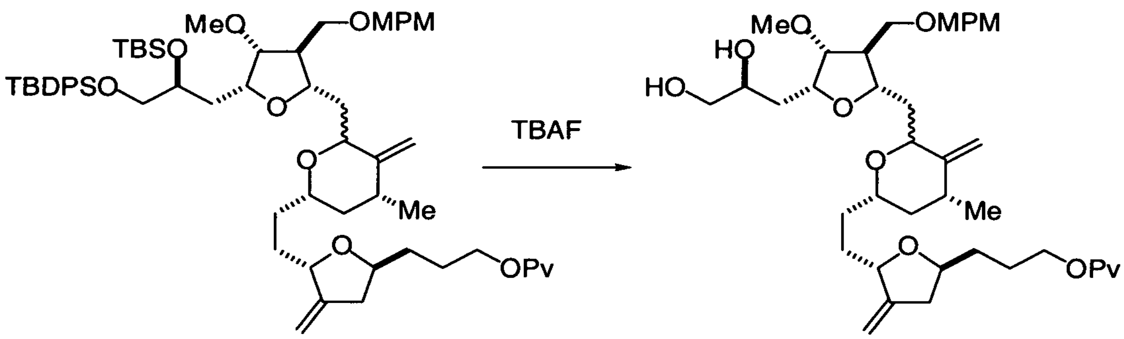
B2090. In a manner similar to that described in Scheme 6 for the synthesis of **B1794**, intermediate **114** was converted to **B2090**. HRMS (FAB): calcd for $C_{39}H_{56}O_{11}$+Na 723.3720. Found: 723.3731.



B2090 **B2136**

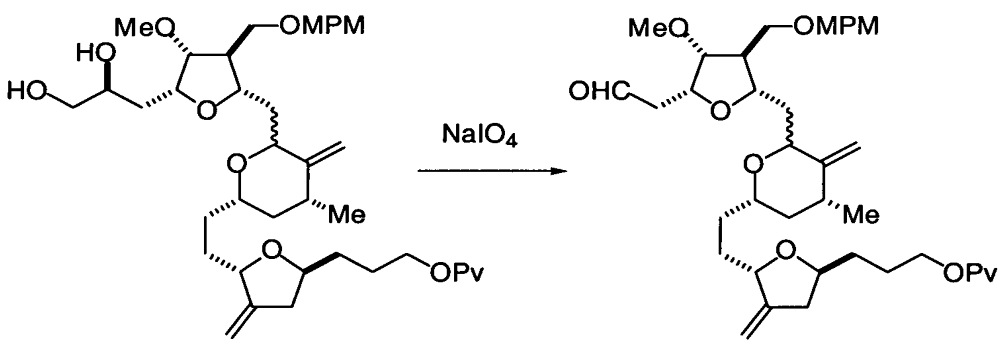
B2136. In a manner analogous to that of **B1939**, **B2090** was converted to **B2136**. HRMS (FAB): calcd for $C_{39}H_{57}NO_{10}$ + Na 722.3880. Found: 722.3907.

Synthesis of B2039/B2043:



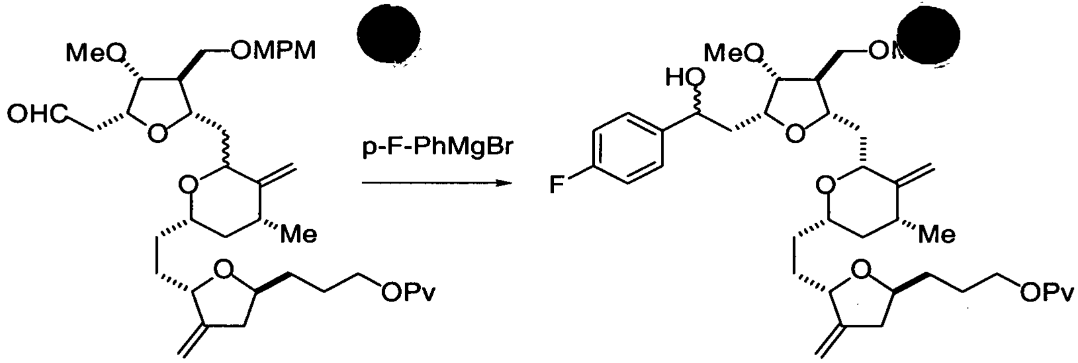
X2318 **201**

Diol 201 TBAF (1 M in THF, 383 μL, 0.383 mmol) was added to a solution of **X2318** **(350-LS-218**)(80.8 mg, 0.0765 mmol) in THF (7 mL) and stirred at rt for 16 h. After partial concentration, the residue was loaded directly onto a SiO_2 column packed using 30% EtOAc-hexanes. Gradient elution (30% EtOAc-hexanes to EtOAc) furnished diol **201** (49.7 mg, 92%).



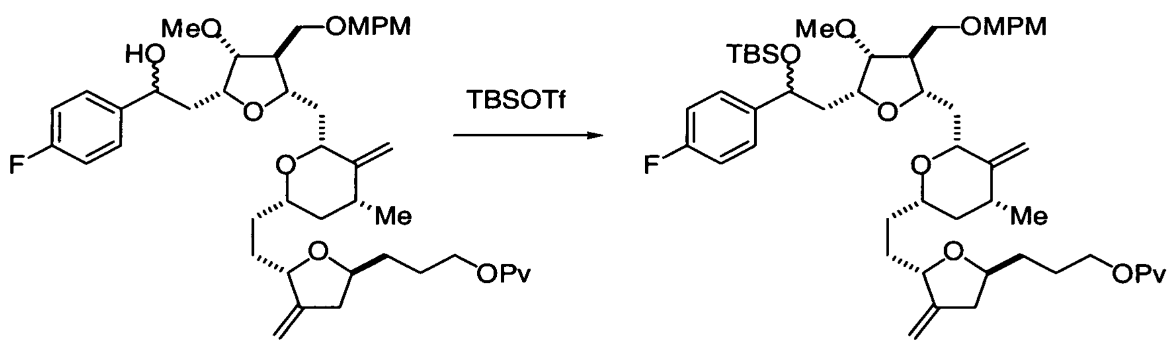
201 **202**

Aldehyde 202 A mixture of diol **201** (49.7 mg, 0.0707 mmol), $NaIO_4$ (100 mg, 0.47 mmol), MeOH (10 mL) and H_2O (2.5 mL) was stirred at rt for 30 min. H_2O was added and the mixture was extracted with CH_2Cl_2 (4×). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide aldehyde **202** (41.7 mg, 88%).



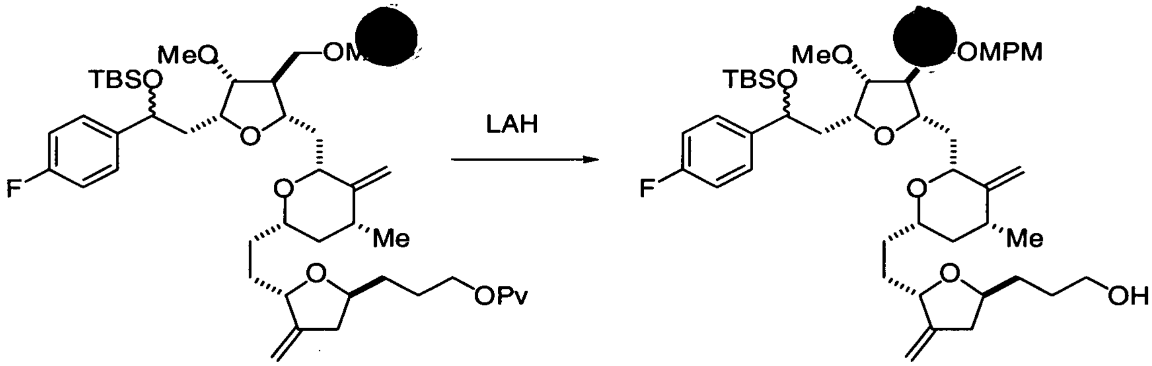
202 **203**

Alcohol 203 4-Fluorophenylmagnesium bromide (2 M in Et_2O, 155 μL, 0.31 mmol) was added to a solution of aldehyde **202** (41.7 mg, 0.062 mmol) in THF (6 mL). After 15 min at rt, the reaction was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (4×). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by preparative TLC (40% EtOAc-hexanes) to provide alcohol **203** (32.4 mg, 68%) as a 1:1 mixture of C34 isomers. The minor undesired C27 isomer was separated at this stage and was also isolated as a 1:1 mixture of C34 isomers (8.4 mg, 18%).



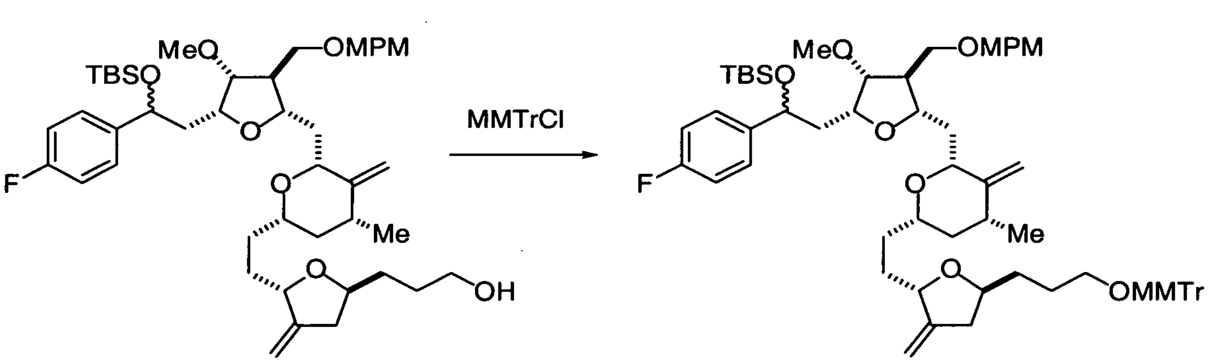
203 **204**

Ether 204 Et_3N (18 μL, 0.13 mmol) and TBSOTf (15 μL, 0.063 mmol) were added to a solution of alcohol **203** (32.4 mg, 0.042 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 20 min the reaction was quenched by the addition of saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3×). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide ether **204** (33.1 mg, 89%).



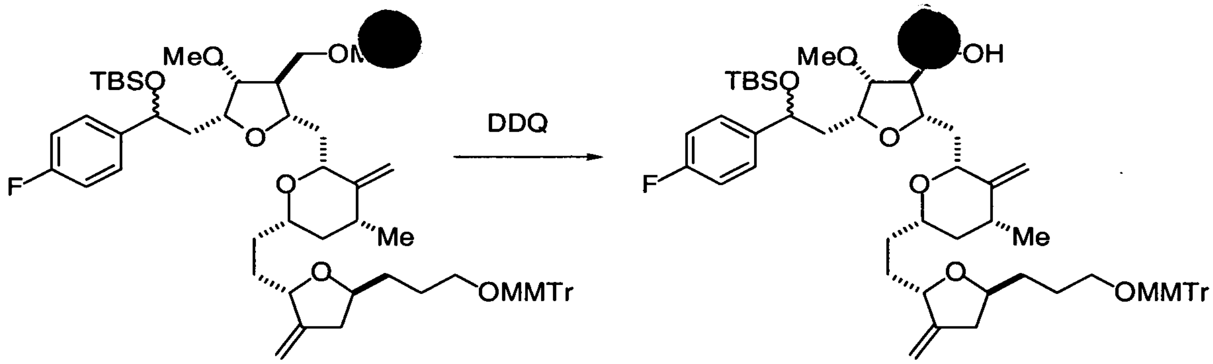
204 **205**

Alcohol 205 LAH (1 M in THF, 113 μL, 0.113 mmol) was added dropwise to a solution of ether **204** (33.1 mg, 0.0375 mmol) in Et_2O (10 mL) at 0 °C. After 20 min, H_2O and 1 M NaOH were added and the mixture was stirred at rt for 10 min. Filtration through Celite, concentration and purification by column chromatography (40% EtOAc-hexanes) furnished alcohol **205** (28.4 mg, 95%).



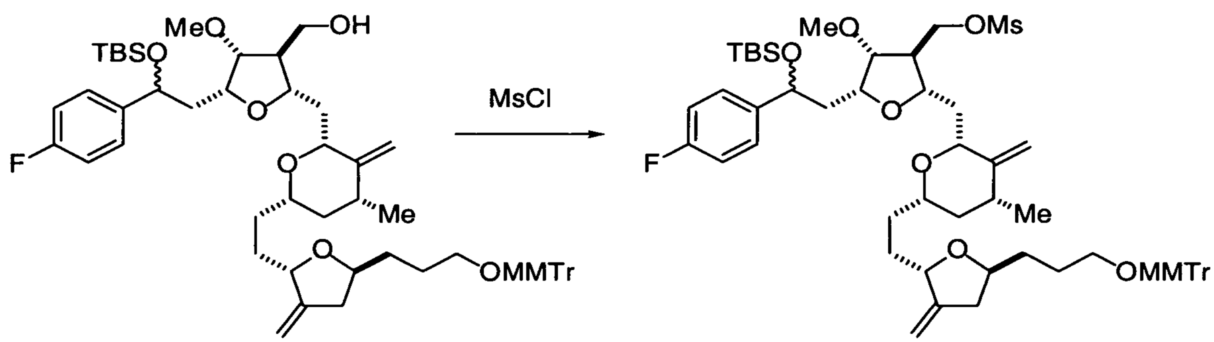
205 **206**

Ether 206 Diisopropylethylamine (31 μL, 0.18 mmol) and MMTrCl (22 mg, 0.071 mmol) were added to a solution of alcohol **205** (28.4 mg, 0.0356 mmol) in CH_2Cl_2 (4 mL) at 0 °C. After 15 h at rt, H_2O was added and the mixture was extracted with CH_2Cl_2 (3×). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (40% EtOAc-hexanes) to provide ether **206** as a ~1.5:1 mixture of C34 epimers (45 mg, quant), which contained a small amount of close-running impurities.



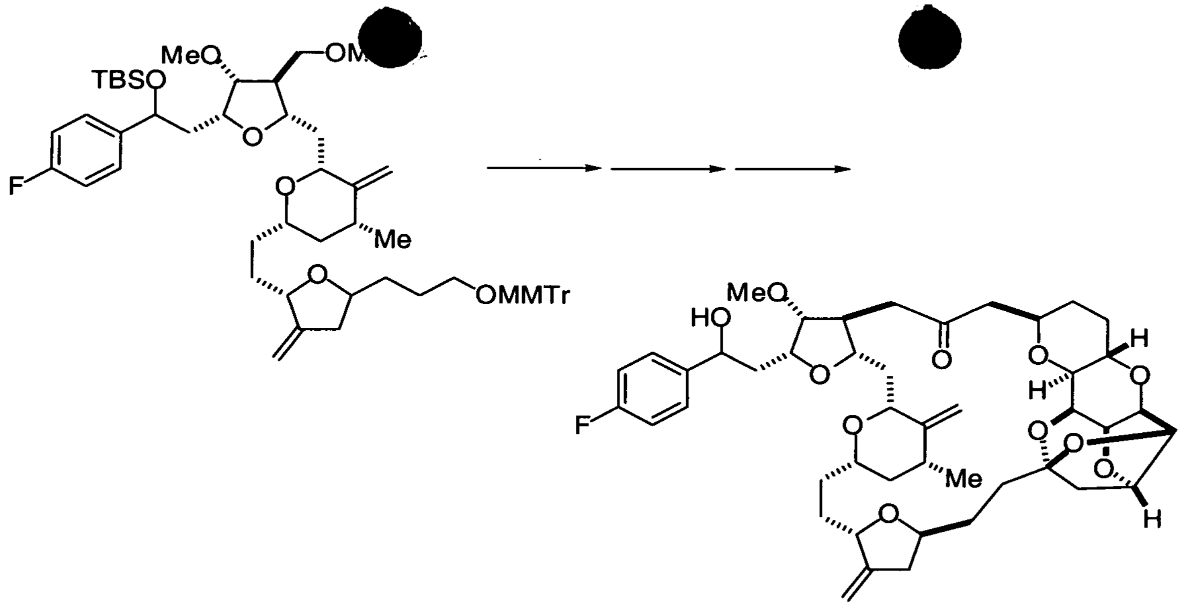
206 **207**

Alcohol 207 DDQ (40 mg, 0.18 mmol) was added to a solution of ether **206** (37 mg, 0.034 mmol) in CH_2Cl_2 (4 mL) and a 1:10 mixture of *t*BuOH:pH 7 phosphate buffer (2 mL) at 0 °C. The mixture was stirred vigorously in the dark for 15 min. Three additional portions of DDQ (40 mg, 0.18 mmol) were added at 10 min intervals, then the reaction was diluted with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide alcohol **207** (19.2 mg, 59%) as well as recovered ether **206** (9.7 mg, 26%).



207 **208A and 208B**

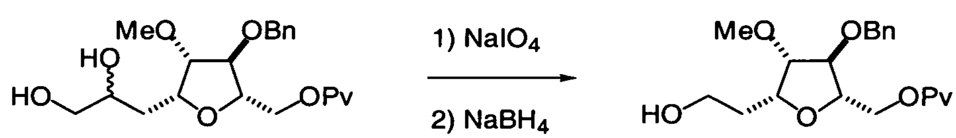
Mesylates 208A and 208B Et_3N (19 μL, 0.13 mmol) and Ms_2O (10 mg, 0.056 mmol) were sequentially added to a solution of alcohol **207** (21.3 mg, 0.022 mmol) in CH_2Cl_2 (6 mL) at 0 °C. After 30 min, saturated aqueous $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (3×). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide mesylates **208A** (11.7 mg, 51%) and **208B** (6.5 mg, 28%) as single C34 isomers.



208A and 208B **B2039, B2043**

B2039 and **B2043**. In a manner similar to that described in Scheme 6 for the synthesis of **B1794**, both diastereomers **208A** and **208B** were independently converted to **B2039** and **B2043**. HRMS (FAB): calcd for $C_{45}H_{59}FO_{11}$ + Na 817.3939. Found: for **B2039** 817.3896, **B2043** 817.3910.

Synthesis of B2086, B2088, B2091

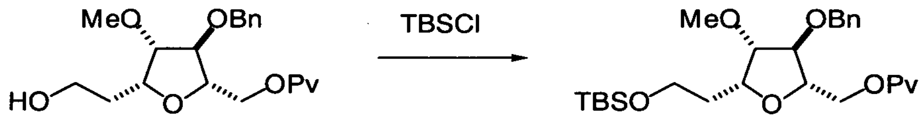


10a, 10b **X- 20**

Alcohol X-20 $NaIO_4$ (1.16 g, 5.4 mmol) was added to a solution of diols **10 a, b** (1.19 g, 3.0 mmol) in MeOH-H_2O (4:1, 75 mL) at 0 °C. The reaction mixture was allowed to warm to rt. After stirring for 40 min, the mixture was diluted with EtOAc, filtered through Celite, concentrated, and partitioned between brine and CH_2Cl_2. The separated aqueous layer was extracted with CH_2Cl_2 (2×). The combined organic layers were dried over Na_2SO_4 and concentrated to furnish the crude aldehyde intermediate.

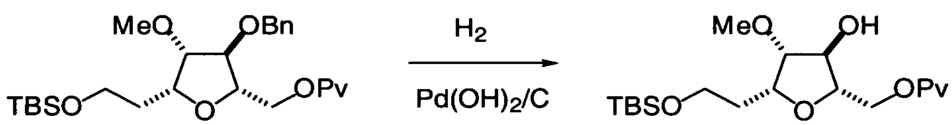
$NaBH_4$ (228 mg, 6.0 mmol) was added to a solution of the aldehyde in MeOH-Et_2O (1:1, 40 mL) at 0 °C. The mixture was stirred for 30 min, carefully quenched with saturated aqueous NH_4Cl, stirred for 20 min at rt and extracted with CH_2Cl_2 (3×). The combined

extracts were dried over Na_2SO_4, concentrated and purified by flash chromatography (40% to 50% EtOAc-hexanes) to afford alcohol **X-20** (1.02 g, 93% for two steps).



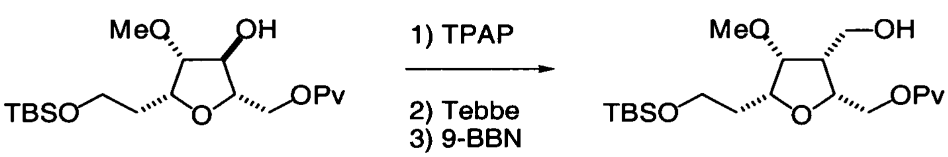
20 **21**

Silyl ether 21 Imidazole (0.94 g, 13.9 mmol) and TBSCl (0.59 g, 3.89 mmol) were added sequentially to a solution of alcohol **X-20** (1.02 g, 2.78 mmol) in DMF (10 mL) at rt. After 14 h, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc (3×). The combined organic extracts were washed with H_2O, brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (5% to 15% EtOAc-hexanes) to afford silyl ether **21** (1.3 g, 98%).



21 **22**

Alcohol 22 A mixture of $Pd(OH)_2$ (20%, 0.8 g), silyl ether **21** (1.3 g, 2.70 mmol) and EtOAc (30 mL) was stirred for 1 h under 1 atm H_2 at rt, filtered through Celite, concentrated and purified by flash chromatography (20% to 40% EtOAc-hexanes) to afford alcohol **22** (0.96 g, 91%).

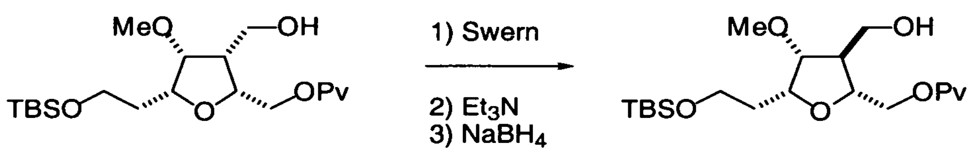


22 **25**

Alcohol 25 4-Methylmorpholine N-oxide (980 mg, 8.4 mmol) and TPAP (131 mg, 3.26 mmol) were added sequentially to a solution of alcohol **22** (1.78 g, 4.6 mmol) in CH_2Cl_2 (45 mL) at rt. A cold bath was necessary to control the exotherm. After 20 min, the reaction mixture was diluted with hexanes, filtered through a short SiO_2 column (15% EtOAc-hexanes) and concentrated to give the crude ketone.

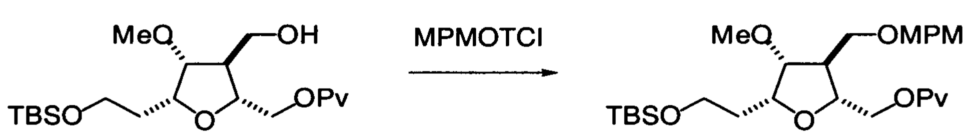
Tebbe reagent (14.9[illegible], 9.0 mmol) was added over 10 min [illegible]olution of the crude ketone in THF (60 mL) at 0 °C. After 20 min, the reaction mixture was poured into Et_2O (100 mL) that was precooled to -78 °C, quenched by slow addition of H_2O (30 mL), warmed to rt., stirred for 30 min and extracted with Et_2O (4×). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (10% EtOAc-hexanes) to afford the desired olefin contaminated by the gem-dimethyl product (1.07 g). This mixture was used directly in the next step.

9-BBN (0.5 M in THF, 11.6 mL, 5.8 mmol) was added to a solution of the olefin in THF (15 mL) at 0 °C. The reaction mixture was allowed to warm to rt, stirred for 5 h and then recooled to 0 °C. H_2O (60 mL), THF (60 mL) and $NaBO_3 \cdot 4\,H_2O$ (5.7 g) were added. After stirring for 5 h at rt, the THF was removed under reduced pressure and the aqueous residue was extracted with EtOAc (4×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (20% to 40% EtOAc-hexanes) to furnish alcohol **25** (605 mg, 18% for three steps).



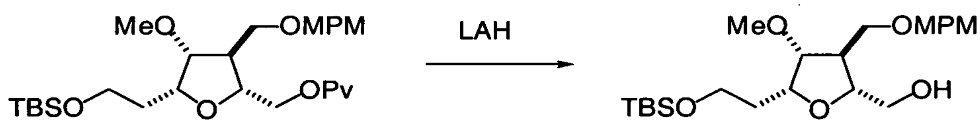
25 **26**

Alcohol 26 Using the procedure previously described, alcohol **25** (604 mg, 1.49 mmol) was sequentially oxidized, isomerized, and reduced. Purification by flash chromatography (20% to 40% EtOAc-hexanes) afforded alcohol **26** (550 mg, 91% for three steps).



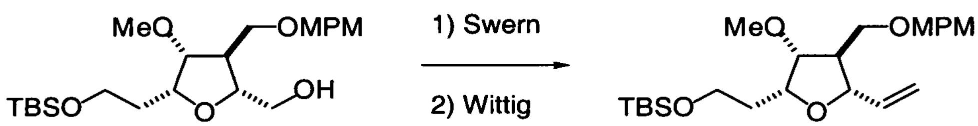
26 **27**

MPM-ether 27 $BF_3 \cdot OEt_2$ (0.05 M in CH_2Cl_2, 270 μL, 0.013 mmol) was added to a solution of alcohol **26** (545 mg, 1.35 mmol) and MPM-trichloroimidate (1.14 g, 4.0 mmol) in CH_2Cl_2 (40 mL) at 0 °C. After 1 h, the reaction was quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2, dried over Na_2SO_4, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford MPM-ether **27** (580 mg, 82%).



27 28

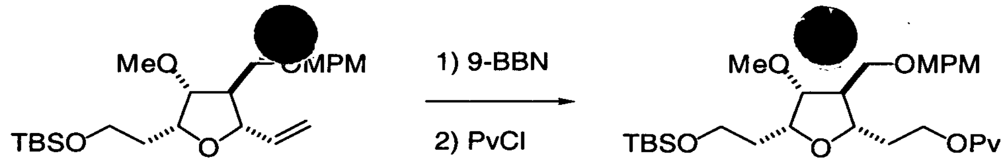
Alcohol 28 LAH (1 M in THF, 1.9 mL, 1.9 mmol) was added to a solution of MPM-ether **27** (580 mg, 1.11 mmol) in Et_2O (100 mL) at 0 °C. After 30 min, the reaction was quenched carefully with H_2O (0.5 mL), and 1 N aqueous NaOH (0.5 mL), stirred for 1 h at rt, filtered through Celite, concentrated and purified by flash chromatography (30% to 50% EtOAc-hexanes) to afford alcohol **28** (460 mg, 95%).



28 29

Olefin 29 DMSO (441 μL, 6.23 mmol) was added to a solution of oxalyl chloride (272 μL, 3.12 mmol) in CH_2Cl_2 (30 mL) at -78 °C. After 15 min, a solution of alcohol **28** (458 mg, 1.04 mmol) in CH_2Cl_2 (15 mL) was added to the reaction mixture. After stirring for 1 h at -78 °C, Et_3N (1.3 mL, 9.35 mmol) was added. The reaction mixture was warmed to 0 °C, stirred for 10 min, diluted with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3×). The combined organic extracts were dried over Na_2SO_4, concentrated and filtered through a short SiO_2 column (20% to 30% EtOAc-hexanes) to provide the crude aldehyde.

n-BuLi (1.63 M, 1.4 mL, 2.28 mmol) was added dropwise to a solution of CH_3PPh_3Br (815 mg, 2.28 mmol), THF (20 mL) and DMSO (7.5 mL) at 0 °C. After 1 h, a solution of the aldehyde in THF (10 mL) was added. The reaction mixture was warmed to rt and stirred for 3 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with EtOAc (4×). The combined organic extracts were washed with H_2O, brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford olefin **29** (380 mg, 95% yield for 2 steps).



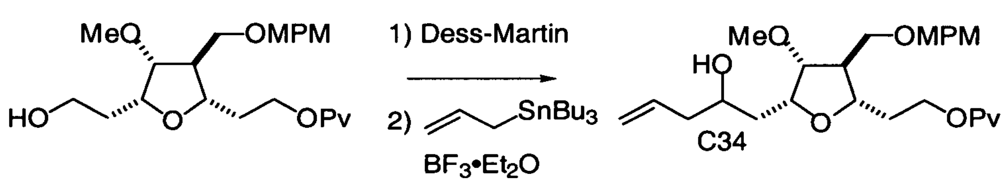
29 **31**

Compound 31 9-BBN (0.5 M in THF, 6 mL, 3 mmol) was added to a solution of olefin **29** (370 mg, 0.85 mmol) in THF (7 mL) at 0 °C. The mixture was allowed to warm to rt and stirred for 1 h. After recooling to 0 °C, H_2O (30 mL), THF (20 mL), and $NaBO_3{\bullet}4\ H_2O$ (2.8 g) were added. After stirring for 3 h at rt, the THF was removed under reduced pressure. The aqueous residue was extracted with EtOAc (4×), dried over Na_2SO_4, concentrated and purified by flash chromatography (25% to 50% EtOAc-hexanes) to afford alcohol **30** which was used directly in the next step.

Pivaloyl chloride (157 μL, 1.27 mmol) was added to a solution of alcohol **30** in CH_2Cl_2-pyridine (1:1 mixture, 10 mL) at rt. After 18 h, additional pivaloyl chloride (100 μL, 0.81 mmol) was added. After 1 h, the reaction mixture was cooled to 0 °C, quenched with MeOH (0.5 mL), concentrated, diluted with brine and extracted with CH_2Cl_2 (4×). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford compound **31** (410 mg, 90% for two steps).

31 **32**

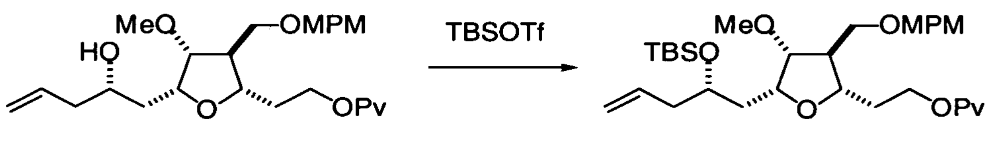
Alcohol 32 TBAF (1 M in THF, 1.14 mL, 1.14 mmol) was added to a solution of **31** (410 mg, 0.761 mmol) in THF (5 mL) at rt. After 1.5 h, the reaction mixture was concentrated and purified by flash chromatography (40% EtOAc-hexanes to 100% EtOAc) to afford alcohol **32** (320 mg, 100%).



32 **33a**: C34 α-OH

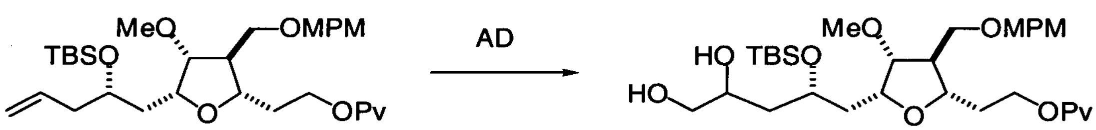
Alcohols 33a and 33b Dess-Martin periodinane (925 mg, 2.18 mmol) was added to a solution of alcohol **32** (309 mg, 0.727 mmol) in CH_2Cl_2 (19 mL) at rt. After 1 h, the reaction was diluted with Et_2O and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous $NaHCO_3$-$Na_2S_2O_3$ and brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (20% to 30% EtOAc-hexanes) to afford the desired aldehyde, which was taken immediately through the next step.

BF_3•OEt_2 (135 μL, 1.1 mmol) was added to a solution of the crude aldehyde, tri-n-butylallyltin (337 μL, 1.08 mmol) and CH_2Cl_2 (16 mL) at -78 °C. After 1 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3×). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by MPLC (25% to 30% EtOAc-hexanes) to afford the major, more polar alcohol **33a** (165 mg, 49% for two steps) and the minor less polar product **33b** (90 mg, 27% for two steps).



33a **34**

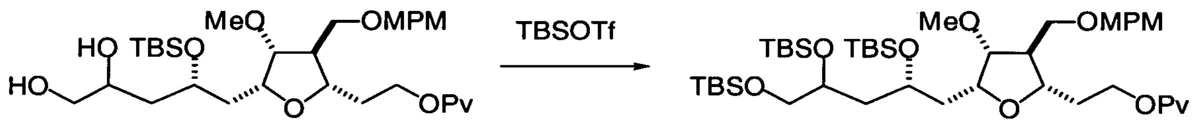
Compound 34. TBSOTf (163 μL, 0.710 mmol) was added to a solution of alcohol **33a** (165 mg, 0.355 mmol), Et_3N (247 μL, 1.78 mmol) and CH_2Cl_2 (5 mL) at 0 °C. After 25 min, the reaction was quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2 (3×), dried over Na_2SO_4, concentrated and purified by flash chromatography (15% to 20% EtOAc-hexanes) to afford compound **34** (200 mg, 98%).



34 **35a and 35b**

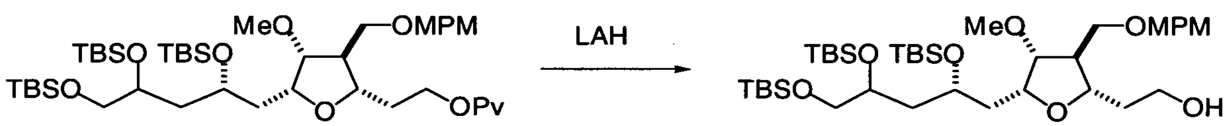
Diols 35a and 35b OsO_4 (0.1 M solution in toluene, 32 μL, 3.2 μmol) was added to a solution of K_2CO_3 (168 mg, 1.22 mmol), $K_3Fe(CN)_6$ (400 mg, 1.22 mmol), $(DHQ)_2PYR$ (11 mg, 12 μmol), H_2O (3.2 mL) and t-BuOH (2.2 mL) at 0 °C. Then a solution of olefin **34** (200 mg, 0.345 mmol) in t-BuOH (1 mL) was added to the reaction mixture. After 5 h at 0 °C, $Na_2S_2O_5$•5 H_2O (200 mg) was added. The reaction mixture was warmed to rt, stirred for 30 min and extracted with CH_2Cl_2 (5×). The combined organic extracts were washed with brine,

dried over Na_2SO_4, concentrated and purified by preparative TLC (70% EtOAc- hexanes) to afford the major, less polar diol **35a** (118 mg, 56%), and minor, more polar diastereomeric product **35b** (74 mg, 35%). The individual diastereomers were each carried forward separately.



35a **36**

Compound 36. TBSOTf (177 μL, 0.77 mmol) was added to a solution of diol **35a** (118 mg, 0.192 mmol), Et_3N (267 μL, 1.92 mmol) and CH_2Cl_2 (5 mL) at 0 °C. After 25 min, the reaction was quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2 (3×), dried over Na_2SO_4, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford compound **36** (161 mg, 100%).



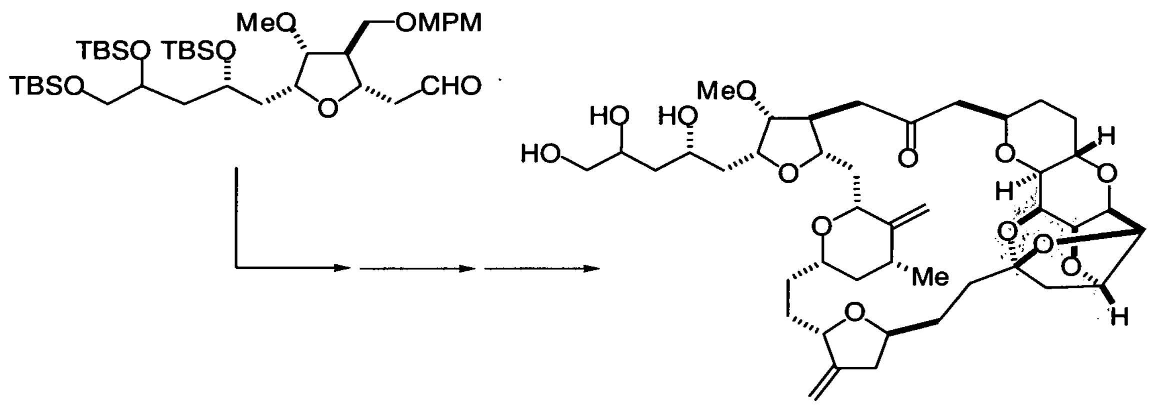
36 **37**

Alcohol 37 Using the procedure described previously for the preparation of alcohol **28**, compound **36** (161 mg, 0.192 mmol) afforded alcohol **37** (135 mg, 93%) after purification by flash chromatography (20% to 40% EtOAc-hexanes).

37 **38**

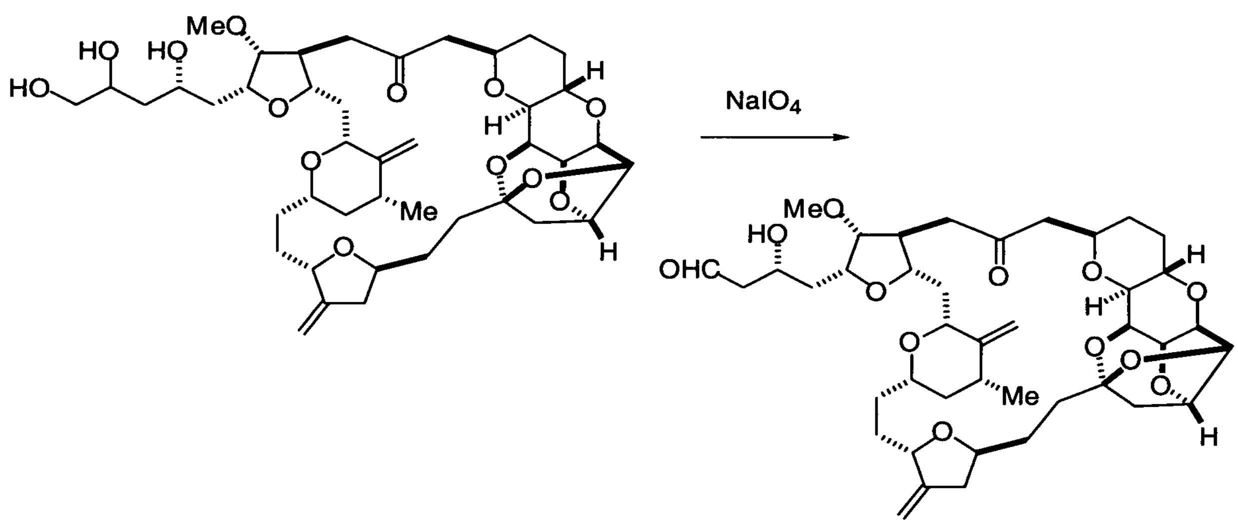
Aldehyde 38 Dess-Martin periodinane (227 mg, 0.535 mmol) was added to a solution of alcohol **37** (135 mg, 0.178 mmol) in CH_2Cl_2 (5 mL) at rt. After 1 h, the reaction mixture was diluted with Et_2O and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous $NaHCO_3$-$Na_2S_2O_3$ and brine, dried over Na_2SO_4,

concentrated and purified by flash chromatography (10% to 20% EtOAc/hexanes) to afford aldehyde **38** (127 mg, 95%).



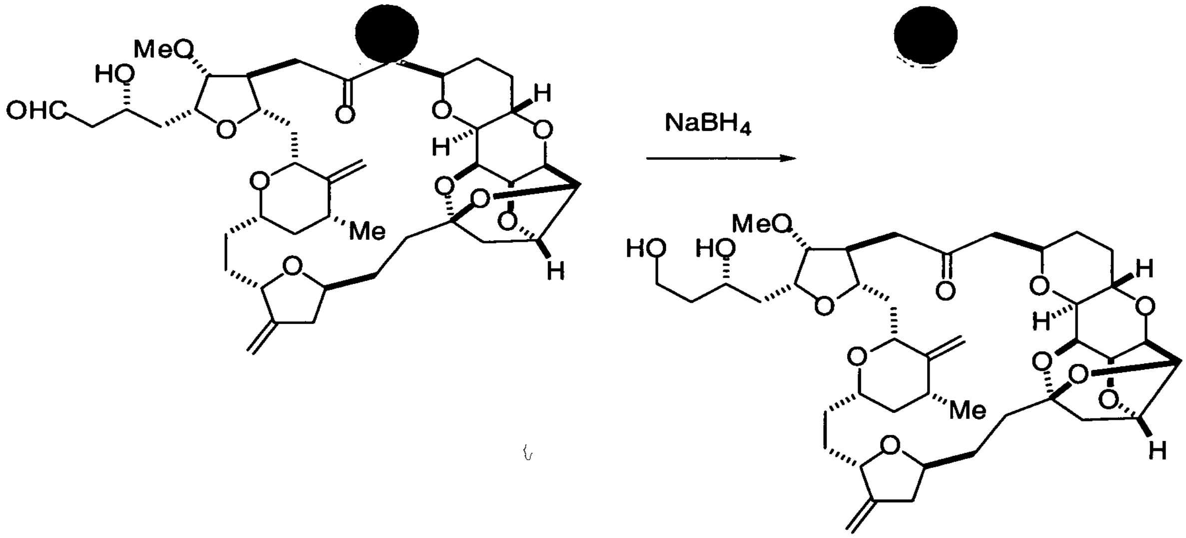
38 **B2086, B2102**

B2086, B2102. Each of the diastereomers obtained above were separately carried to final product in a manner similar to that described in scheme 6 for **B1794**. Diastereomer **35a** afforded **B2086**. Diastereomer **35b** afforded **B2102**.



B2086 **B2088**

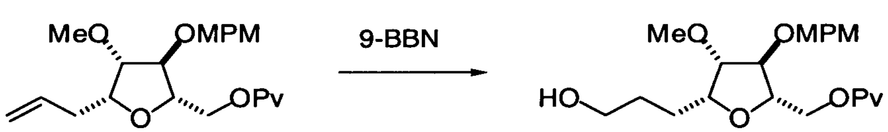
B2088 $NaIO_4$ was added to a solution of **B2086** (1 mg, 1.29 μmol) in MeOH-H_2O (4:1,
1 mL) at rt. After 30 min, the reaction mixture was diluted with H_2O, extracted with CH_2Cl_2 (6×), dried over Na_2SO_4, and concentrated to afford **B2088** (1.2 mg).



B2088 **B2091**

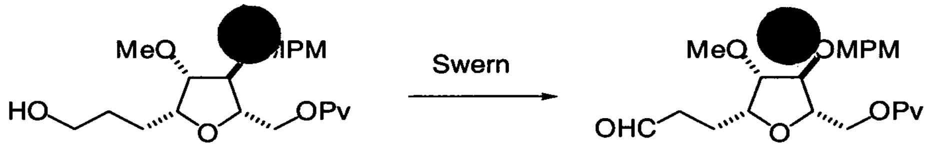
B2091 $NaBH_4$ (0.013 M in EtOH, 20 μL, 0.27 μmol) was added to a solution of **B2088** (1 mg, 1.29 μmol) in MeOH-CH_2Cl_2 (4:1, 0.5 mL) at -78 °C. Additional $NaBH_4$ was periodically added with close monitoring of the reaction by TLC (total of 220 μL of the $NaBH_4$ solution was required). The reaction mixture was quenched at 0 °C with saturated aqueous NH_4Cl, stirred for 20 min at rt and extracted with CH_2Cl_2 (6×). The combined extracts were dried over Na_2SO_4, concentrated and purified by preparative TLC (7% MeOH-EtOAc) to furnish **B2091** (0.40 mg, 50%).

Synthesis of B1933:



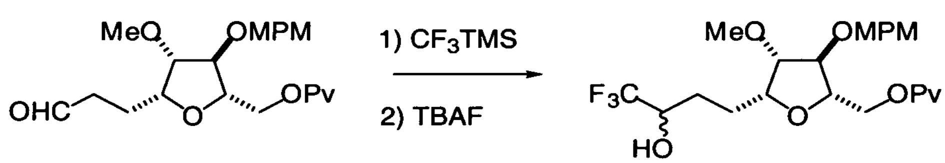
302 **303**

Alcohol 303 9-BBN (0.5 M in THF, 23 mL, 0.012 mol) was added dropwise over 30 min to a solution of alkene **302** (1.51 g, 0.00386 mol) in THF (40 mL) at 0 °C. After stirring at rt for 80 min, the mixture was cooled to 0 °C and H_2O (80 mL) was cautiously added followed by $NaBO_3$•4 H_2O (4.2 g, 0.027 mol). The mixture was stirred vigorously at rt for 2.3 h, then extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol **303** (1.37 g, 87%).



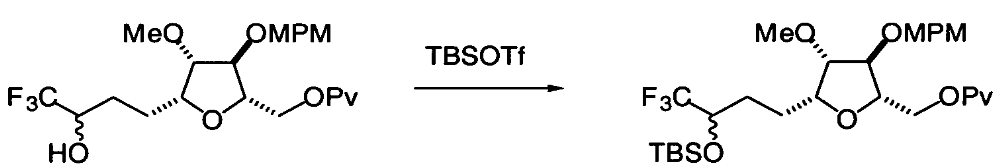
303 **304**

Aldehyde 304 Oxalyl chloride (88 μL, 1.00 mmol) was added dropwise to a solution of DMSO (142 μL, 2.00 mmol) in CH_2Cl_2 (20 mL) at -78 °C. After 30 min, a solution of alcohol **303** (137 mg, 0.335 mmol) in CH_2Cl_2 (5 mL) was added and stirred at -78 °C for 1 h. Et_3N (420 μL, 3.01 mmol) was added and after 10 min the reaction was stirred for 10 min at 0 °C at which point saturated aqueous NH_4Cl was added and the resulting mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (50% EtOAc-hexanes) to provide intermediate aldehyde **304** (0.114 g, 84%) which was immediately used in the next step.



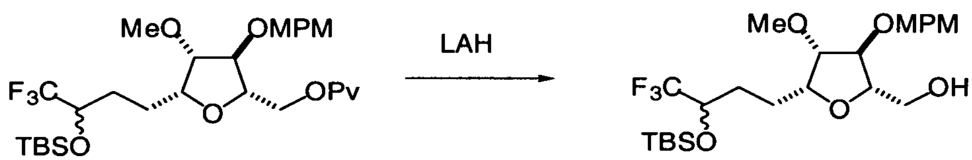
304 **305**

Alcohol 305 TBAF (1 M in THF, 5 μL, 0.005 mmol) was added to a solution of aldehyde **304** (0.114 g, 0.27 mmol) in CF_3TMS (0.5 M in THF, 1.1 mL, 0.54 mmol) at 0 °C. After 20 min, a second portion of TBAF (1 M in THF, 100 μL, 0.1 mmol) was added and the mixture was stirred for 10 min at which point excess TBAF (1 M in THF, 270 μL, 0.27 mmol) was added dropwise to cleave the intermediate silyl ether. After 30 min, the mixture was diluted with H_2O and extracted with EtOAc (3×). The organic extracts were washed with H_2O, brine, dried over Na_2SO_4, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol **305** (123 mg, 95%) as an inseparable 1:1 mixture of isomers.



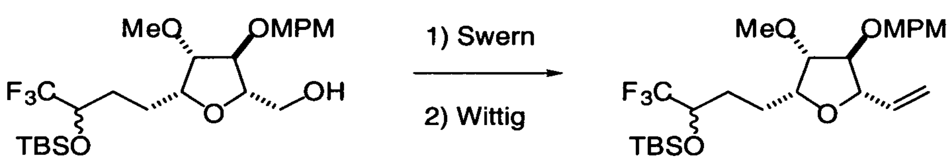
305 **306**

Silyl ether [illegible] TBSOTf (265 μL, 1.16 mmol) wa[illegible]ded to a solution of alcohol **305** (123 mg, 0.257 mmol) and Et_3N (430 μL, 3.08 mmol) in CH_2Cl_2 (8 mL) at 0 °C. After stirring at rt for 20 h, saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide silyl ether **306** (148 mg, 97%).



306 **307**

Alcohol 307 LAH (1 M in THF, 220 μL, 0.22 mmol) was added dropwise to a solution of silyl ether **306** (131 mg, 0.22 mmol) in Et_2O (5 mL) at 0 °C. After 20 min, H_2O and 1 M NaOH were cautiously added. The mixture was stirred at rt 30 min, filtered through glass wool, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol **307** (112 mg, quant.).

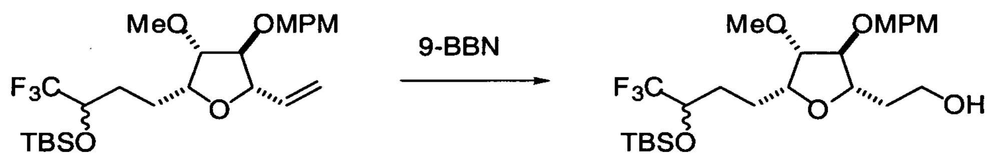


307 **309**

Alkene 309 Oxalyl chloride (58 μL, 0.66 mmol) was added dropwise to a solution of DMSO (94 μL, 1.3 mmol) in CH_2Cl_2 (10 mL) at -78 °C. After 30 min, a solution of alcohol **307** (112 mg, 0.22 mmol) in CH_2Cl_2 (3 mL) was added. After 1 h, Et_3N (276 μL, 1.98 mmol) was added, and after 10 min at -78 °C the reaction was stirred at 0 °C for 10 min. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (50% EtOAc-hexanes) to provide aldehyde **308** (101 mg, 91%) which was immediately used in the next step.

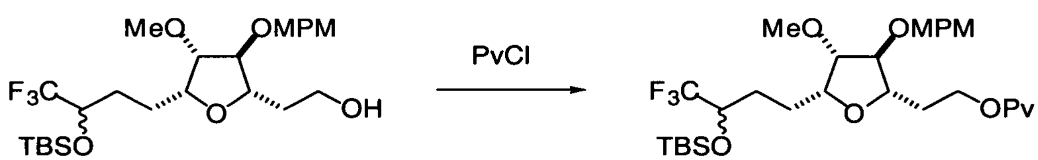
*n*BuLi (1.63 M in THF, 200 μL, 0.33 mmol) was added dropwise to a solution of CH_3PPh_3Br (118 mg, 0.33 mmol) in THF (3 mL) and DMSO (1.2 mL) at 0 °C. After 70 min, a solution of aldehyde **308** (101 mg, 0.20 mmol) in THF (3 mL) was added and after 10 min at

0 °C, the reaction was stirred at rt for 1 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alkene **309** (90.9 mg, 90%).



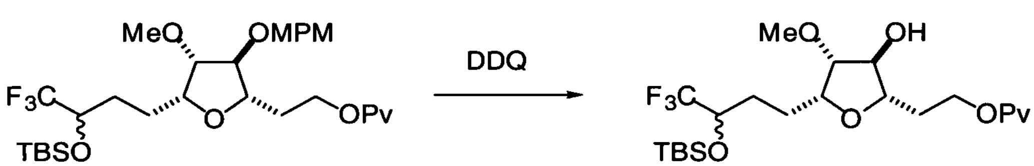
309 **310**

Alcohol 310 9-BBN (0.5 M in THF, 17 mL, 8.45 mmol) was added dropwise to a solution of alkene **309** (1.06 g, 2.11 mmol) in THF (30 mL) at 0 °C. After stirring for 2.5 h at rt, the reaction was cooled to 0 °C and H_2O (60 mL) followed by $NaBO_3$•4 H_2O (3.25 g, 21.1 mmol) were cautiously added. The mixture was stirred vigorously at rt for 2 h, then diluted with H_2O and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol **310** (0.920 g, 84%).



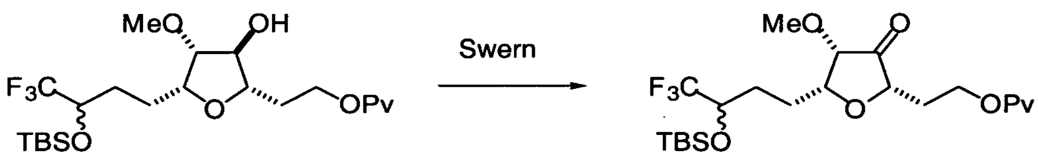
310 **311**

Pivaloate 311 A mixture of alcohol **310** (65.8 mg, 0.0126 mmol), pyridine (61 μL, 0.76 mmol) and PvCl (23 μL, 0.189 mmol) in CH_2Cl_2 (3 mL) was stirred at rt for 5 h. A second reaction utilizing alcohol **310** (0.92 g, 1.76 mmol) was run under similar conditions and both reactions were combined during the work-up: saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide pivaloate **311** (1.08 g, quant).



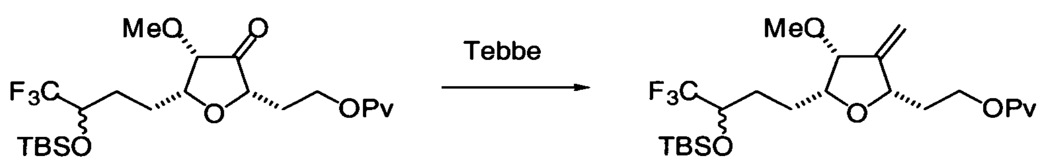
311 312

Alcohol 312 A mixture of ether **311** (0.811 g, 1.33 mmol), DDQ (6.1 g, 27 mmol) and 10:1 *t*BuOH: pH 7 phosphate buffer (42 mL) in CH_2Cl_2 (84 mL) was stirred vigorously in the dark at rt for 1.5 h, at which point additional DDQ (1.0 g, 4.4 mmol) was added. After 1 h, saturated aqueous $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (4×). The combined organic extracts were washed successively with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alcohol **312** (0.56 g, 87%) as well as recovered starting material **311** (97 mg, 12%).



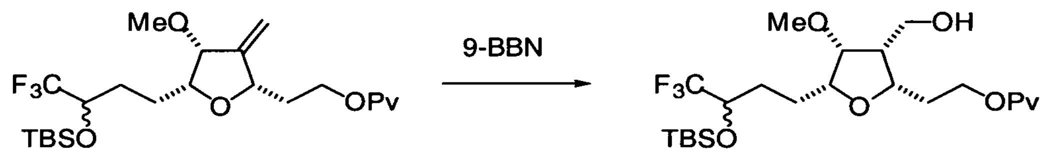
312 313

Ketone 313 Oxalyl chloride (21 μL, 0.12 mmol) was added dropwise to a solution of DMSO (34 μL, 0.48 mmol) in CH_2Cl_2 (3 mL) at -78 °C. After 1 h, a solution of alcohol **312** (39.4 mg, 0.081 mmol) in CH_2Cl_2 (1.5 mL) was added and the mixture was stirred for 1.5 h. Et_3N (100 μL, 0.73 mmol) was added, and after 10 min the mixture was warmed to 0 °C. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide ketone **313** (36.6 mg, 93%) which was used immediately in the next step.



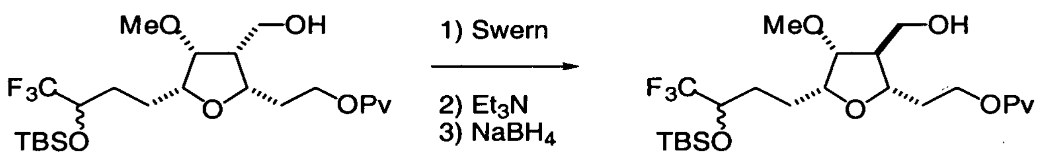
313 314

Alkene 314 Tebbe reagent (~0.65 M in toluene, 720 μL, 0.47 mmol) was added dropwise to a solution of ketone **313** (151 mg, 0.31 mmol) in THF (5 mL) at 0 °C. After 15 min, H_2O was cautiously added and the mixture was extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (10% EtOAc-hexanes) to provide alkene **314** (139 mg, 93%).



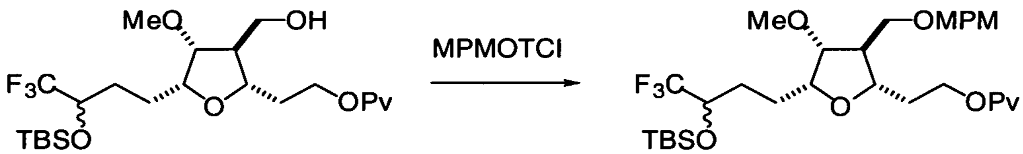
314 **315**

Alcohol 315 9-BBN (0.5 M in THF, 6.0 mL, 2.9 mmol) was added dropwise to a solution of alkene **314** (468 mg, 0.97 mmol) in THF (10 mL) at 0 °C. The mixture was stirred at rt for 2 h at which point additional 9-BBN (0.5 M in THF, 500 μL, 0.25 mmol) was added. After 2.5 h, the mixture was cooled to 0 °C and H_2O (10 mL) followed by $NaBO_3 \cdot 4\ H_2O$ (1.5 g, 9.7 mmol) were cautiously added. The mixture was stirred vigorously at rt for 5 h, diluted with H_2O and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (gradient 20% to 30% EtOAc-hexanes) to provide alcohol **315** (0.47 g, 97%).



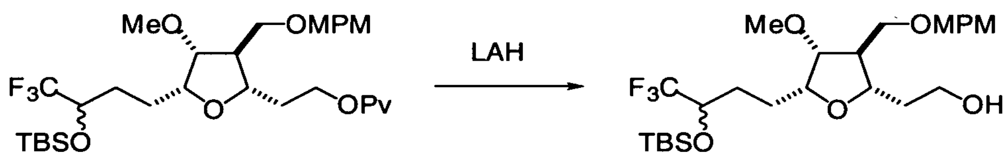
315 **316**

Alcohol 316 Oxalyl chloride (246 μL, 2.82 mmol) was added dropwise to a solution of DMSO (400 μL, 5.64 mmol) in CH_2Cl_2 (40 mL) at -78 °C. After 1 h, a solution of alcohol **315** (0.47 g, 0.94 mmol) in CH_2Cl_2 (10 mL) was added and the mixture was stirred for 1 h. Et_3N (1.2 mL, 8.5 mmol) was added, and after 10 min the mixture was warmed to 0 °C and stirred for 10 min. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The crude aldehyde was stirred in CH_2Cl_2 (20 mL) and Et_3N (2 mL) at rt overnight. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (30% EtOAc-hexanes) provided the epimerized aldehyde which was immediately dissolved in 1:1 Et_2O:EtOH (10 mL) and cooled to 0 °C. $NaBH_4$ (35 mg, 0.94 mmol) was added and after 10 min the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with EtOAc (3×) and the combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol **316** (0.410 g, 87% yield for 3 steps).



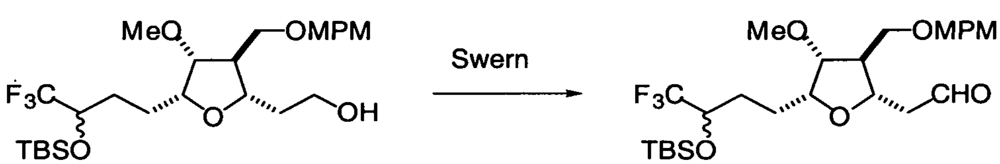
316 **317**

Ether 317 Alcohol **316** (60.7 mg, 0.12 mmol) and MPMOTCI (0.10 g, 0.36 mmol) were combined, azeotroped from toluene (3×) and dried under high vacuum overnight. CH_2Cl_2 (3 mL) was added and the mixture was cooled to 0 °C. BF_3•OEt_2 (approx. 1 μL, 0.01 mmol) was added and after stirring for 10 min the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2 (3×) and the combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide ether **317** (55.4 mg, 74%).



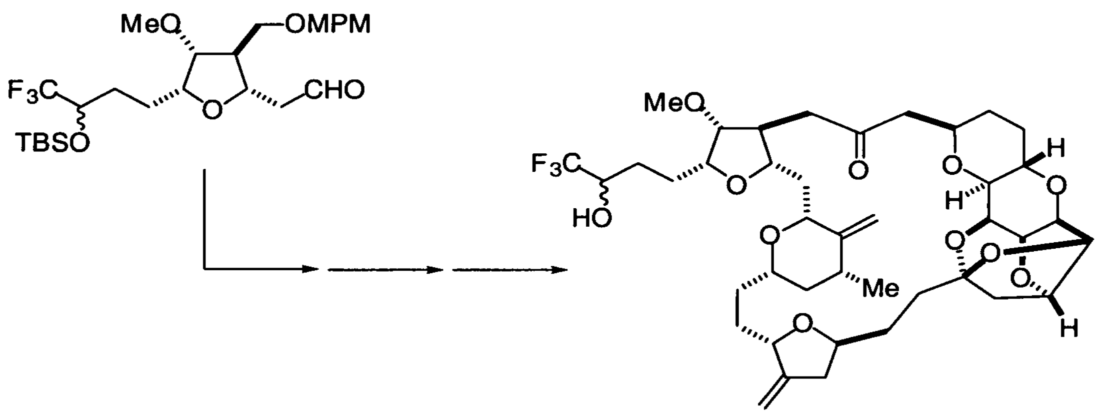
317 **318**

Alcohol 318 LAH (1 M in THF, 104 μL, 0.104 mmol) was added dropwise to a solution of ether **317** (54 mg, 0.087 mmol) in Et_2O (5 mL) at 0 °C. After 30 min, H_2O and 1 M NaOH were cautiously added. The mixture was stirred at rt for 10 min, filtered through glass wool, concentrated and purified by column chromatography (30%-50% EtOAc-hexanes) to provide alcohol **318** (45.5 mg, 98%).



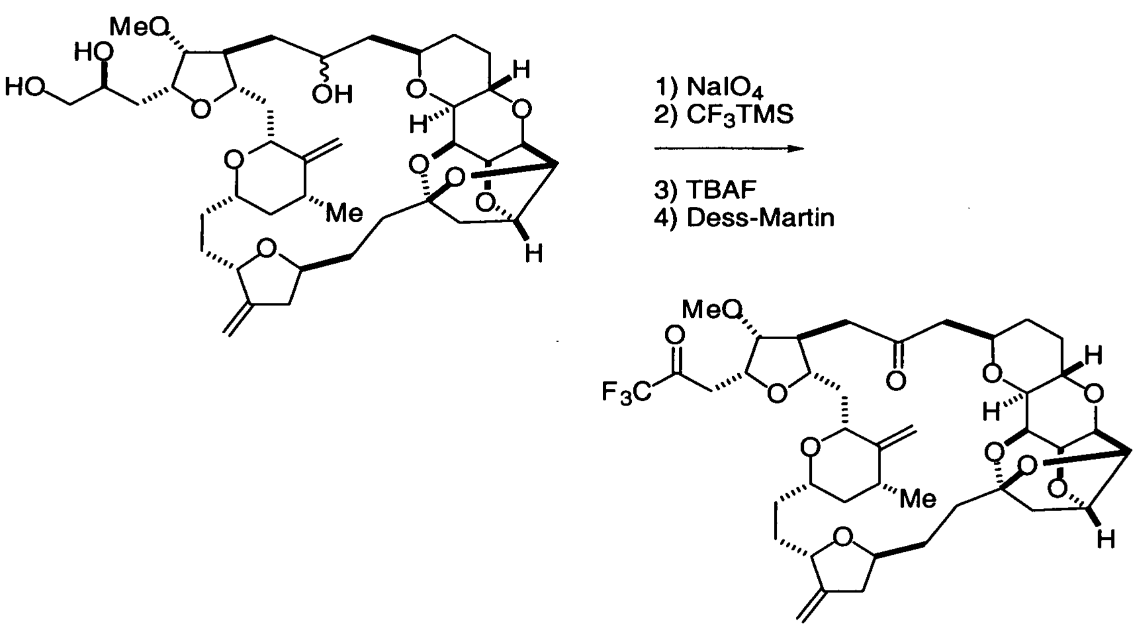
318 **319**

Aldehyde 319 Oxalyl chloride (11 μL, 0.13 mmol) was added dropwise to a solution of DMSO (18 μL, 0.25 mmol) in CH_2Cl_2 (2 mL) at -78 °C. After 1.8 h, a solution of alcohol **318** (22.6 mg, 0.042 mmol) in CH_2Cl_2 (1 mL) was added and the mixture was stirred for 1 h. Et_3N (53 μL, 0.38 mmol) was added and after 10 min, the reaction was warmed to 0 °C and stirred 10 min. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to provide aldehyde **319** (21.7 mg, 97%).



319 **B1933**

B1933. In a manner similar to that described in Scheme 6 for the synthesis of **B1794**, intermediate **319** was converted to **B1933**. HRMS (FAB): calcd for $C_{41}H_{57}F_3O_{11}$ + H 783.3931. Found: 783.3940.



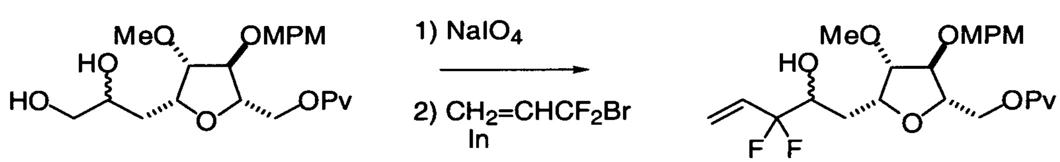
B1942. A mixture of **B1897** (2 mg, 2.73 μmol), $NaIO_4$ (35 mg, 0.16 mmol), MeOH (0.8 mL) and H_2O (0.2 mL) was stirred at rt for 30 min. The reaction mixture was then diluted with H_2O (3 mL) and extracted with CH_2Cl_2 (6×) and EtOAc (2×). The combined organic phases were dried over Na_2SO_4 and purified by column chromatography (5% MeOH- CH_2Cl_2) to give the desired aldehyde.

This material was dissolved in THF (0.1 mL), cooled to at 0 °C and treated with 0.5 M CF_3TMS in THF (30 μL, 15 mmol) followed by 0.05 M TBAF in THF (5 mL, 0.025 mmol). After stirring for 30 min, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (2 mL) and H_2O (1 mL), extracted with EtOAc (6×), dried over Na_2SO_4, filtered and concentrated to give the crude bis-TMS ether.

This material was dissolved in THF (0.5 mL) and treated with 1 M TBAF in THF containing 0.5 M imidazole hydrochloride (8 μL, 8 μmol) at rt for 30 min. The reaction mixture was eluted through a SiO_2 column (50% EtOAc-hexanes to EtOAc) to afford the diol intermediate.

A mixture of this product and Dess-Martin periodinane (10 mg, 24 mmol) in CH_2Cl_2 (0.5 mL) was stirred at rt for 1 h, diluted with Et_2O (5 mL) and filtered through Celite. The filtrate was concentrated and purified by preparative TLC (50% EtOAc-hexanes) to furnish **B1942** (1.5 mg, 72% for 5 steps). HRMS (FAB): calcd for $C_{40}H_{55}F_3O_{11}$ + H 767.3516. Found: 767.3542

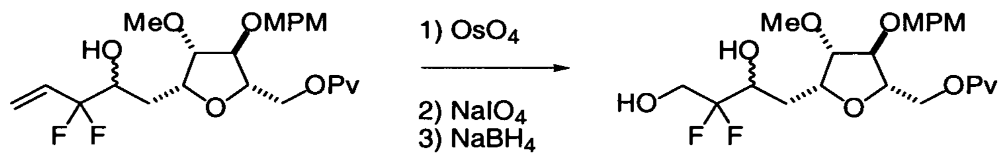
Synthesis of B2070/B2073:



X400 **401**

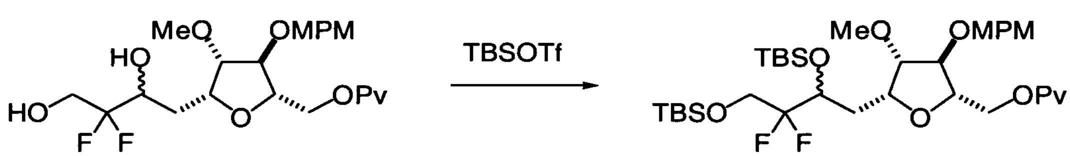
Alcohol 401 A mixture of $NaIO_4$ (375 mg, 1.74 mmol), **X400** (674 mg, 1.58 mmol), MeOH (16 mL) and H_2O (4 mL) was stirred at rt for 1 h. After dilution with H_2O, the mixture was extracted with CH_2Cl_2 (4×) and the combined organic extracts were dried over Na_2SO_4, concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide the intermediate aldehyde (570 mg), which was immediately dissolved in DMF (15 mL). Indium (275 mg, 2.4 mmol) and 3-bromo-3,3-difluoropropene (240 μL, 2.4 mmol) were added and after stirring at rt for 17 h, H_2O and 0.1 M HCl were added. The mixture was extracted with EtOAc (3×) and the combined organic extracts were washed successively with H_2O and brine,

dried over Na_2SO_4, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol **401** as a 1:1 mixture of C34 isomers (605 mg, 81% for 2 steps).



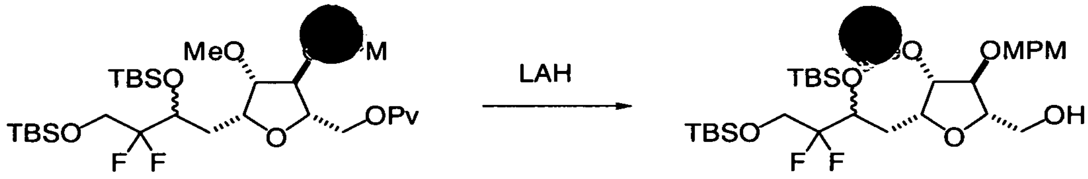
401 **402**

Diol 402 A mixture of OsO_4 (1 xstal), alcohol **401** (605 mg, 1.28 mmol), 4-methylmorpholine *N*-oxide (0.45 g, 3.84 mmol), acetone (30 mL) and H_2O (6 mL) was stirred at rt for 29 h. Additional OsO_4 (3 xtals) and 4-methylmorpholine *N*-oxide (0.1 g, 0.8 mmol) were added and after 2 days saturated aqueous $Na_2S_2O_3$ was added. The mixture was extracted with CH_2Cl_2 (6×) and the combined organic extracts were dried over Na_2SO_4 and concentrated. The crude intermediate triol was immediately dissolved in 4:1::MeOH:H_2O (25 mL) and $NaIO_4$ (0.41 g, 1.9 mmol) was added. After stirring vigorously at rt for 2 h, the mixture was diluted with H_2O, extracted with CH_2Cl_2 (3×) and the combined organic extracts were dried over Na_2SO_4 and concentrated to provide the intermediate aldehyde which was immediately dissolved in 1:1 EtOH-Et_2O (30 mL) and cooled to 0 °C. $NaBH_4$ (48 mg, 1.3 mmol) was added and after 20 min the reaction was quenched with H_2O and extracted with CH_2Cl_2 (4×). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide diol **402** (485 mg, 80% for 3 steps).



402 **403**

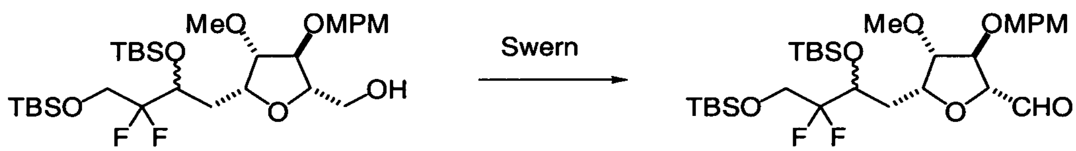
Silyl ether 403 TBSOTf (2.3 mL, 10 mmol) was added dropwise to a mixture of diol **402** (485 mg, 1.0 mmol), Et_3N (2.8 mL, 20 mmol) and CH_2Cl_2 (30 mL) at 0 °C. After stirring for 1 h at rt, saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide silyl ether **403** (668 mg, 95%).



403 4

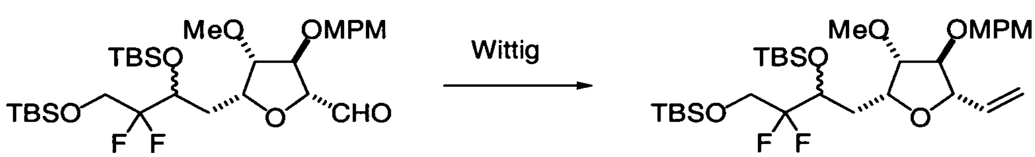
04

Alcohol 404 LAH (1 M in THF, 2.8 mL, 2.8 mmol) was added dropwise to a solution of silyl ether **403** (668 mg, 0.948 mmol) in Et_2O (60 mL) at 0 °C. After 15 min, H_2O and 1 M NaOH were cautiously added. The mixture was stirred at rt for 20 min, filtered through glass wool, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol **404** (500 mg, 85%).



404 **405**

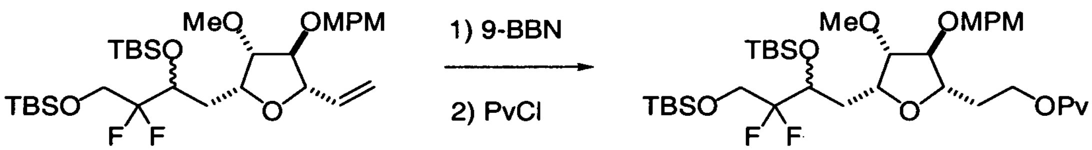
Aldehyde 405 Oxalyl chloride (210 μL, 2.42 mmol) was added dropwise to a solution of DMSO (345 μL, 4.84 mmol) in CH_2Cl_2 (30 mL) at -78 °C. After 1 h, a solution of alcohol **404** (500 mg, 0.806 mmol) in CH_2Cl_2 (10 mL) was added. After 40 min, Et_3N (1.0 mL, 7.2 mmol) was added. After stirring at -78 °C for 10 min, the reaction mixture was warmed to 0 °C and stirred for an additional 10 min. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed successively with H_2O, brine, dried over Na_2SO_4 and concentrated. Purification by flash chromatography (30% EtOAc-hexanes) provided aldehyde **405** (486 mg, 98%) which was immediately used in the next step.



405 **406**

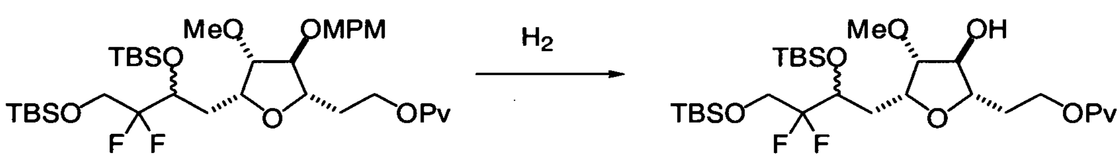
Alkene 406 *n*BuLi (1.63 M, 860 μL, 1.4 mmol) was added dropwise to a solution of CH_3PPh_3Br (500 mg, 1.4 mmol) in THF (15 mL) and DMSO (6 mL) at 0 °C. After 1 h, a solution of aldehyde **405** (486 mg) in THF (15 mL) was added. The reaction mixture was warmed to rt and stirred for 30 min. Saturated aqueous NH_4Cl was added, the mixture was

extracted with EtOAc (3×) and the combined extracts were washed successively with H_2O and brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alkene **406** (450 mg, 93%).



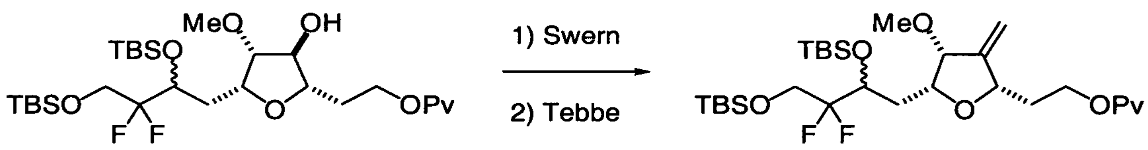
406 **407**

Ester 407 9-BBN (0.5 M in THF, 9.0 mL, 4.5 mmol) was added dropwise to a solution of alkene **406** (0.460 g, 0.746 mmol) in THF (10 mL) at 0 °C. After warming to rt, the mixture was stirred for 3 h and two additional portions of 9-BBN (0.5 M in THF, 3.0 mL, 1.5 mmol) were added at 30 min intervals. The reaction mixture was recooled to 0 °C, whereupon THF (10 mL), H_2O (10 mL) and $NaBO_3$•4 H_2O (1.72 g, 11.2 mmol) were cautiously added. The mixture was stirred vigorously at rt for 1.5 h, and additional $NaBO_3$•4 H_2O (1.0 g, 6.5 mmol) was added. After 2 h the mixture was diluted with H_2O and extracted with EtOAc (3×). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide the intermediate alcohol (509 mg) which was immediately dissolved in CH_2Cl_2 (10 mL) and treated with pyridine (600 μL, 7.5 mmol) and PvCl (275 μL, 2.2 mmol). After 6 h, saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide ester **407** (423 mg, 79% for 2 steps).



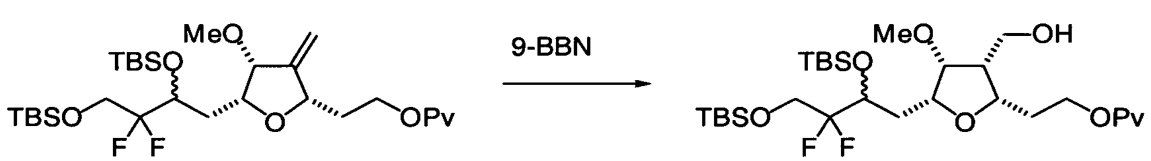
407 **408**

Alcohol 408 A mixture of ester **407** (11 mg, 0.015 mmol) and $Pd(OH)_2/C$ (10 mg) in EtOAc (500 μL) was stirred vigorously under a H_2 atmosphere at rt for 6 h. The mixture was filtered through Celite, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol **408** (9.4 mg, quant).



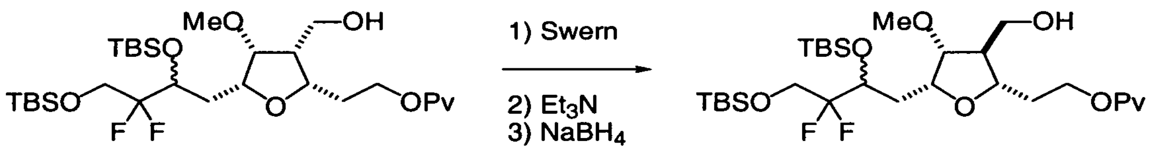
408 **409**

Alkene 409 Oxalyl chloride (7 μL, 0.075 mmol) was added dropwise to a solution of DMSO (11 μL, 0.15 mmol) in CH_2Cl_2 (2 mL) at -78 °C under N_2. After 40 min, a solution of alcohol **408** (15.2 mg, 0.025 mmol) in CH_2Cl_2 (1 mL) was added and the reaction was stirred at -78 °C for 1 h. Et_3N (31 μL, 0.22 mmol) was added, and after stirring for 10 min the mixture was warmed to 0 °C. After 10 min, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3×). The combined extracts were washed successively with H_2O and brine, dried over Na_2SO_4 and concentrated. After flash chromatography (30% EtOAc-hexanes), the intermediate ketone (13 mg) was immediately dissolved in THF (500 μL) and treated with Tebbe reagent (~0.65 M in toluene, 62 μL, 0.040 mmol) at 0 °C. After 1.5 h additional Tebbe reagent (~0.65 M in toluene, 62 μL, 0.040 mmol) was added and after 10 min H_2O and then brine were cautiously added. The mixture was extracted with EtOAc (3×) and the combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (10% EtOAc-hexanes) to provide alkene **409** (11.9 mg, 80% for 2 steps).



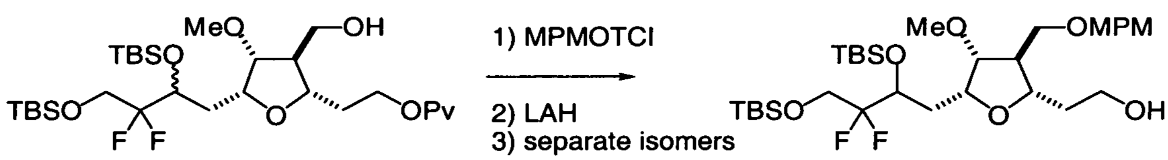
409 **410**

Alcohol 410 9-BBN (0.5 M in THF, 1.5 mL, 0.72 mmol) was added dropwise to a solution of alkene **409** (0.144 g, 0.242 mmol) in THF (2 mL) at 0 °C. After warming to rt, the mixture was stirred for 3 h. The reaction mixture was recooled to 0 °C, whereupon THF (2 mL), H_2O (2 mL) and $NaBO_3 \cdot 4\ H_2O$ (0.38 g, 2.4 mmol) were cautiously added. The mixture was stirred vigorously at rt for 4 h, diluted with H_2O and extracted with EtOAc (3×). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alcohol **410** (0.140 g, 94%).



410 **411**

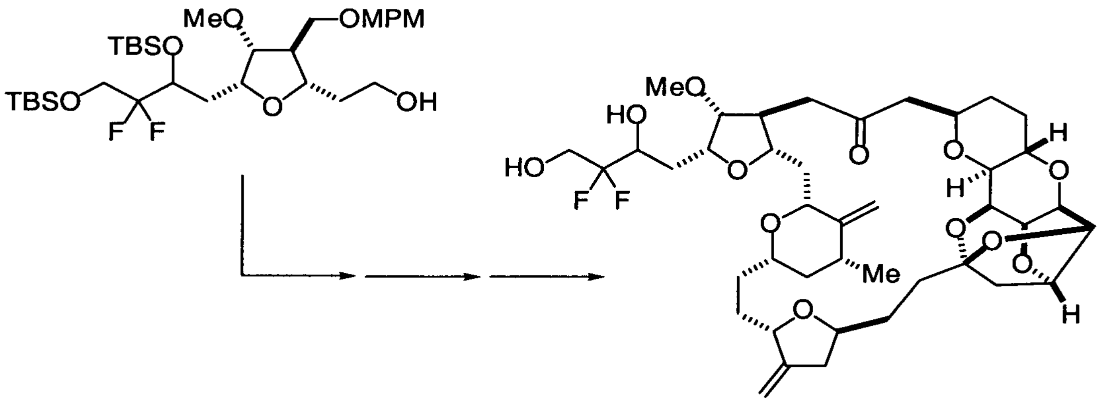
Alcohol 411 Oxalyl chloride (26 μL, 0.30 mL) was added dropwise to a solution of DMSO (43 μL, 0.60 mmol) in CH_2Cl_2 (4 mL) at -78 °C. After 1 h, a solution of alcohol **410** (57 mg, 0.093 mmol) in CH_2Cl_2 (2 mL) was added. After 45 min, Et_3N (125 μL, 0.90 mmol) was added. After stirring at -78 °C for 10 min, the reaction mixture was warmed to 0 °C and stirred for an additional 10 min. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. The crude product was dissolved in CH_2Cl_2 (4 mL), treated with Et_3N (400 μL) and stirred at rt for 15 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide the intermediate aldehyde (48 mg), which was immediately dissolved in 1:1 Et_2O-EtOH (4 mL), cooled to 0 °C and treated with solid $NaBH_4$ (~4 mg, 0.09 mmol). After stirring for 15 min, saturated aqueous NH_4Cl was cautiously added and the mixture was extracted with EtOAc (3×). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol **411** (45.6 mg, 80% for 3 steps).



411 **412A, 412B**

412A and 412B Alcohol **411** (120 mg, 0.196 mmol) and MPMOTCI (0.17 g, 0.59 mmol) were combined, azeotroped from toluene (3×) and dried under high vacuum for 1 h. CH_2Cl_2 (9 mL) was added and the mixture was cooled to 0 °C. BF_3•OEt_2 (0.016 M in CH_2Cl_2, 125 μL, 0.002 mmol) was added dropwise and after stirring for 20 min, the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2 (3×) and the combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (20% EtOAc-hexanes) to provide the intermediate MPM ether which contained some close-running impurities. This material was immediately dissolved in Et_2O (10 mL) and treated with LAH (1M in THF, 300 μL, 0.300 mmol) at 0 °C. After 10 min, H_2O and 1 M

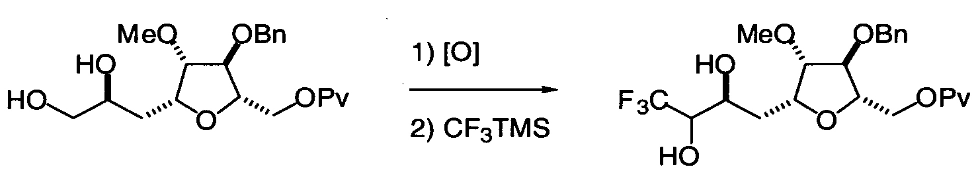
NaOH were added, and after stirring for 10 min at rt, the mixture was filtered through Celite, concentrated and purified by preparative TLC (35% EtOAc-hexanes) to provide **412A** (49 mg, 39% for 2 steps) as a single C34 isomer and **412B** (46 mg, 36% for 2 steps) as a ~9:1 mixture of C34 isomers.



412A, 412B **B2070, B2073**

B2070 and B2073. In a manner similar to that described in Schemes 4 and 6 for the synthesis of **B1794**, intermediates **412A** and **412B** were converted to **B2070** and **B2073**, respectively. For **B2070**: HRMS (FAB): calcd for $C_{41}H_{58}F_2O_{12}$ + Na 803.3794. Found: 803.3801. For **B2073**: HRMS (FAB): calcd for $C_{41}H_{58}F_2O_{12}$ + Na 803.3793. Found: 803.3781

Synthesis of B1963:

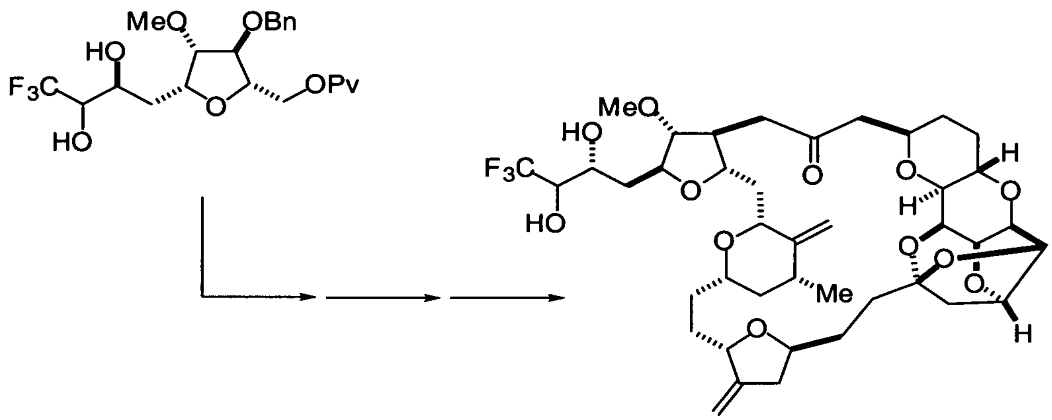


10 **501**

Diol 501 (64) Saturated aqueous $NaHCO_3$ (21 mL) and KBr (89 mg, 0.75 mmol) were added to a solution of diol **10** (1.35 g, 3.4 mmol) in CH_2Cl_2 (34 mL). The mixture was

cooled to 0 °C, and 4-meth[illegible]-2,2,6,6-tetramethyl-1-piperidinyloxy[illegible].05 M in CH_2Cl_2, 7.45 mL, 0.37 mmol) and NaOCl (0.07 M in H_2O, 5.6 mL, 0.39 mmol) were sequentially added. After 1 h, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$, diluted with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3×). The combined extracts were dried over Na_2SO_4, concentrated and dissolved in THF (21 mL).

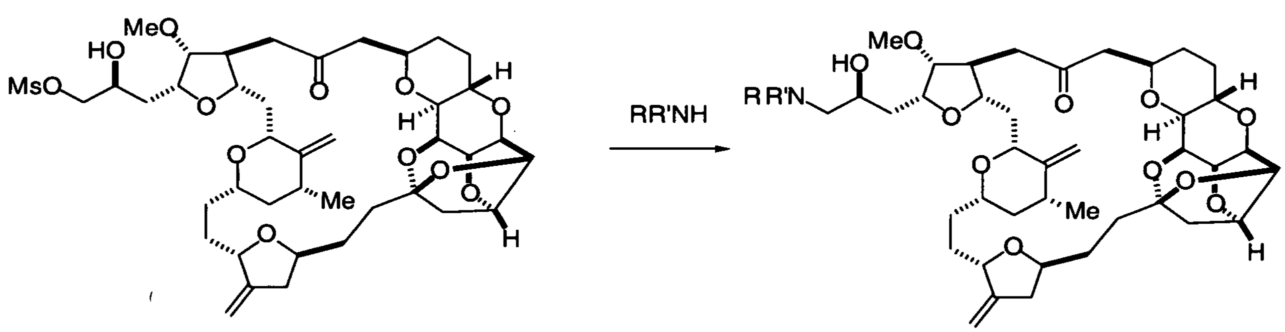
After cooling to 0 °C, CF_3TMS (1.5 g, 10.5 mmol) and TBAF (0.1 M in THF, 680 μL, 0.068 mmol) were sequentially added. After stirring for 40 min, additional TBAF (1 M in THF, 8.3 mL, 8.3 mmol) was added. After 30 min, the reaction was quenched with H_2O and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (30%, 40%, 50% EtOAc-hexanes followed by EtOAc) to afford a 2:1 mixture of diols (553 mg, 35%). Separation by MPLC (1.5% MeOH-CH_2Cl_2) gave the major, more polar isomer **501 (64)** (340 mg, 22%) and the minor, less polar isomer (152 mg, 10%).



B1963

B1963. In a manner similar to that described in Schemes 4 and 6 for the synthesis of **B1794**, intermediate **501** was converted to **B1963**.

Synthesis of B2320 and Related Analogs

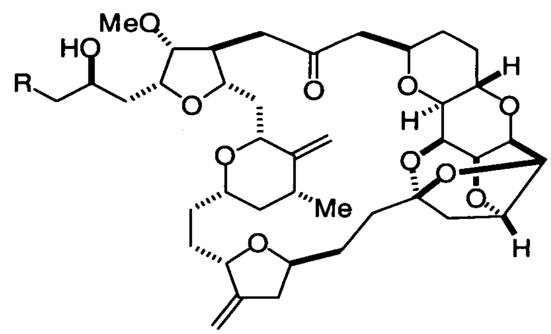


B2294

These compounds are made by treating B2294 with an appropriate amine in a solvent such as methanol for a period of a few hours to several days. Progress of the reaction may be monitored by thin layer chromatography. A standard work-up procedure, well known to those of skill in the art, provides the desired compounds. The procedure below is to prepare ER803868; however this procedure is general and can be used to prepare any desired analog.

Synthesis of ER803868

To a solution of B2294, 1.2 mg, in methanol, 0.5 mL, was added morpholine, 0.012 mL. The mixture was stirred for 10 days with additional morpholine, 0.012 mL, being added on days 1,2,3, 4 and 8. The mixture was then chromatographed to give 1.4 mg of the desired compound.



B2320	R = N,N-dimethylamino
B2330	R = N-isopropylamino
B2336	R = N-methylamino
B2339	R = N-t-butylamino
B2417	R = N-2-hydroxyethylamino
B2418	R = N-piperazinyl
B2489	R = N,N-bis-(2-hydroxyethyl)amino
B2490	R = N-1,3-dihydroxy-2-propylamino
B2491	R = N-benzylamino
ER803834	R = N-piperidinyl
ER803835	R = N-pyrrolidinyl
ER803836	R = N-3-(R)-hydroxypyrrolidinyl
ER803843	R = N-homopiperidinyl
ER803845	R = N-para-methoxybenzylamino
ER803846	R = N-phenethylamino
ER803851	R = N-2-(S-hydroxymethyl)pyrrolidinyl
ER803852	R = N-2-(R-hydroxymethyl)pyrrolidinyl
ER803868	R = N-morpholinyl
ER803869	R = N-ethylamino

ER803870 R = N-imidazoyl

ER803883 R = N,N-diethylamino

ER803884 R = N-para-chlorobenzylamino

D. Pharmacological Activity

Many of the individually disclosed drugs were tested for *in vitro* and *in vivo* activity (see Table 1, below). Screening methods included a standard *in vitro* cell growth inhibition assay using DLD-1 human colon cancer cells (ATCC accession number CCL 221) in a 96-well microtiter plate format (Finlay, G.J. et al Analytical Biochemistry 139:272-277, 1984), a U937 (ATCC accession number CRL 1593) mitotic block reversibility assay (described below), and in some cases, a LOX human melanoma tumor xenograft *in vivo* growth inhibition assay (see Table 1). Chemical stability to esterase degradation was also examined.

U937 Mitotic Block Reversibility Assay

U937 human histiocytic lymphoma cells were added to 75 cm^2 tissue culture flasks as 2.5 x 10^6 cells in 22.5 mL of RPMI Medium 1640 containing 10% Fetal Bovine Serum. Cells were allowed to adapt to the culture during 36 h of incubation at 37 °C in a humidified atmosphere containing 5% CO_2. Each test drug was then added to a flask as 2.5 mL of 10x final concentration. Final concentrations achieved were 0.1-1000 nM, in half log-increments, for a total of 10 concentration steps including a drug-free control flask which recieved 2.5 mL of media. Cells were incubated with drug for 12 h pretreatment period at 37 °C in a humidified atmosphere containing 5% CO_2.

The contents were removed from each flask and centrifuged at 300 x g for 10 min at room temperature, after which drug-containing media was removed from cell pellet. Cells were resuspended in 25 mL of warm drug-free media and centrifuged at 300 x g for 10 min at room temperature. After removing media from cell pellet, cells were resuspended in 35 mL of warm drug-free media, transferred to fresh flasks, and a 10 mL sample of cells immediately removed from each flask, immediately processed as described below and stored for later cell cycle analysis (0 hours of drug washout).

Incubation of the remaining 25 mL of cells continued in drug-free media for another 10 h. A 10 mL sample of cells was removed from each flask, immediately processed and stored for later cell cycle analysis (10 hours of drug washout) and 10 mL fresh replacement media was added to each incubation flask. Incubation of cells in drug-free media continued for 5 days. At day two, 20 mL of media and cells was removed from each flask and replaced with 20 mL fresh media. Viability of cells was quantified after 5 days by trypan blue exclusion techniques using hemacytometer counting.

Cells were processed for cell cycle analysis using modifications of the method published in Becton Dickinson Immunocytometry Systems source book section 1.11 (Preparation of Alcohol-Fixed Whole Cells From Suspensions For DNA Analysis). Briefly, each 10 mL sample of cells removed from the flasks at 0 and 10 hours of drug washout was separately centrifuged at 300 x g for 10 min. After removing the media from the cell pellet, cells were resuspended in 3 mL cold saline. Seven milliliters cold 100 % ethanol was slowly added with vigorous vortexing. Ethanol treated cell samples from 0 hour and 10 hour periods of compound washout were stored overnight at 4 °C. Ethanol treated cells were centrifuged 300 x g for 10 min, ethanol removed and cells then washed in 10 mL Phosphate Buffered Saline (PBS). Cells were resuspended in 0.5 mL of 0.2 mg/mL Ribonuclease A (Sigma No. R-5503) in PBS and incubated in 37 °C water bath for 30 min.

Cells were transferred to appropriate flow cytometry tubes and 0.5 mL of 10 mg/mL propidium iodide (PI) (Sigma No. P4170) in PBS was added to each tube. Cells were incubated with PI at room temperature in the dark for at least 15 min prior to analysis with a flow cytometer (Becton Dickinson FACScan flow cytometer or equivalent). Cells should be analyzed within an hour and kept in the dark at 4 °C until ready. Cell cycle analysis was performed on 0 hour and 10 hour cells using flow cytometric measurement of the intensity of cellular fluorescence. The intensity of propidium iodide fluorescence for each cell was measured on a linear amplification scale with doublet events ignored using doublet discrimination. The results obtained from analyzing 15,000 cells were presented as a histogram with increasing fluorescence intensity on the x-axis and the number of cells at a particular intensity level on the y-axis.

The intensity of PI staining is dependent on the amount of DNA in the cell so it is possible to identify cells in various phases of the cell cycle, such as cells that have not yet synthesized DNA since the last mitosis (G_1 phase), cells that are in intermediate stages of DNA synthesis (S phase), and cells that have doubled their complement of DNA and are ready to divide (G_2 phase). Cells that are blocked in the mitosis phase of the cell cycle also have double the amount of DNA compared to G_1 phase cells. If all cells are blocked in mitosis there are no G_1 phase cells, but if the block is removed when compound is removed, cells complete mitosis and reappear in the G_1 phase. The number of cells so reappearing in the G_1 or Sphase is thus a measure of the number of cells which have recently completed mitosis. For each sample at 0 and 10 hours after compound removal, the percentage of cells completing mitosis was quantified (as the number of cells reappearing in the G_1 phase) and plotted as a function of the initial concentration of compound used during the 12 hour pretreatment period. The percentage of cells still viable 5 days after drug washout was superimposed on the same graph, see, for example FIG. 1 and FIG. 2. A ratio can be determined between the compound concentration required to completely block all cells in mitosis at 0 hour and the concentration required to maintain the block 10 hours after compound removal. This was taken as a measure of a

compound's reversibility, with ratios close to or equal to one indicating likely potent *in vivo* anti-tumor compounds (see Table 1, columns 4-6, and FIGS. 3 and 4).

Table 1

In Vitro Inhibition and Reversibility Data

	DLD-1*		Complete Mitotic Block		Reversibility
compound	mean IC50, nm	SE	0 hour, nM**	10 hour, nM‡	Ratio
B1793	0.93	0.04	3	44	14.7
B1794	12.20	0.72			
B1918	1.27	0.12			
B1920	2.00	0.15			
B1921	24.00	1.15			
B1922	0.53	0.01	3	30	10.0
B1930	0.87	0.03			
B1933	0.79	0.16			
B1934	1.05	0.21	3	30	10.0
B1939	19.34	2.36	12	12	1.0
B1940	5.43	0.62			
B1942	0.60	0.03	3	30	10.0
B1963	0.56	0.04	3	20	6.7
B1973	1.15	0.24			
B1984	1.01	0.15			
B1987	1.82	0.21			
B1988	2.67	1.02			
B1990	1.30	0.06			
B1991	0.69	0.03			
B1992	0.86	0.07			
B1998	1.23	0.13			
B2003	1.21	0.12			
B2004	0.63	0.04			
B2008	2.63	0.63			
B2010	0.71	0.12			
B2011	1.81	0.52			
B2013	0.49	0.07	2	30	15.0
B2014	0.87	0.09			
B2015	2.78	0.23			
B2016	0.66	0.06			
B2019	0.82	0.07			
B2034	0.74	0.03			
B2035	0.76	0.09			

B2037	0.66	0.11			
B2039	0.91	0.08			
B2042	1.93	0.11	3	100	33.3
B2043	1.70	0.06			
B2070	0.64	0.09	3	30	10.0
B2073	0.89	0.15			
B2086	11.17	1.96			
B2088	1.23	0.12			
B2090	0.52	0.04	2	10	5.0
B2091	1.36	0.07	3	30	10.0
B2102	3.47	0.22			
B2136	5.23	1.04	3	3	1.0
B2294	0.80	0.01			
B2320	1.20	0.17	1	10	10.0
B2330	4.40	0.42	7	7	1.0
B2336	3.33	0.09	10	10	1.0
B2339	4.30	0.21	3	3	1.0
B2417	12.67	0.33	10	10	1.0
B2418	3.63	0.17	10	10	1.0
B2489	14.67	2.03	10	10	1.0
B2490	35.67	3.33	100	100	1.0
B2491	0.92	0.14	2	10	6.7
ER803834	0.47	0.08	1	10	10.0
ER803835	15.33	0.33	10	10	1.0
ER803836	1.97	0.12	3	10	3.3
ER803843	0.49	0.05	1	10	10.0
ER803845	1.50	0.20	3	10	3.3
ER803846	1.16	0.10	1	10	10.0
ER803851	3.33	0.26	3	3	1.0
ER803852	3.03	0.50	3	10	3.3
ER803868	0.43	0.03	3	10	3.3
ER803869	4.13	0.64	3	3	1.0
ER803870	1.27	0.12	3	30	10.0
ER803883	1.02	0.04	1	10	10.0
ER803884	0.59	0.03	1	10	10.0

* = in vitro cell growth inhibition ** = before washout ‡ = after washout

The invention also features a method for identifying an agent that induces a sustained mitotic block in a cell after transient exposure of the cell to the agent. The invention features determining the relative reversibility of the test compound by relating the measurement of step (d) and the measurement of step (f), as described below. This determination may be a ratio, or

an arithmetic difference, for example. In one aspect, the method includes:

(a) incubating a first cell sample with a predetermined concentration of a test compound for a time interval between that sufficient to empty the G_1 population and that equivalent to one cell cycle (e.g., typically, 8 –16 hours, or about 12 hours);

(b) substantially separating the test compound from said first cell sample (e.g. by washing or changing media);

(c) incubating said first sample in media free of the test compound for a time interval sufficient to allow at least 80% (e.g., 85%, 90%, and preferably 95%, 98%, or 99%) of the cells released from the mitotic block induced by a highly reversible mitotic inhibitor to complete mitosis and return to the G_1 phase (e.g., typically 6-14 hours, or about 10 hours after separation step (b)); and

(d) measuring the percentage of transiently-exposed cells from step (c) that have completed mitosis and returned to the G_1 phase (e.g., measuring a cell cycle marker, such as DNA-dependent PI fluorescence).

One aspect of this screening method include the further steps of :

(e) incubating a second sample of cells with a concentration of the test compound less than or equal to that used in step (a) for a time interval between that sufficient to empty the G_1 population and that equivalent to one cell cycle;

(f) measuring the percentage of cells from step (e) that have completed mitosis and have returned to the G_1 phase; and

(g) determining a reversibility ratio of the test compound.

In one embodiment of the method, the first and second cell samples are suspension culture cells selected from, for example, human leukemia, human lymphoma, murine leukemia, and murine lymphoma cells. The first and second cell samples may be incubated simultaneously (steps (a) and (e)) or in separate portions. Other embodiments further include before step (a), the step (i) of estimating a desirable time interval for incubating said first cell sample with a reversible mititotic blocking agent (or, alternatively, said test compound) to provide a satisfactory majority of cells collected at mitotic block; and wherein the incubation of step (a) is for the time interval estimated in step (i). Another embodiment of the method further includes before step (c), the step (ii) of estimating a desirable time interval for the test compound-free incubation of step (c), said step (ii) comprising determining the time interval after which at least 80 % of the cells pretreated with a highly reversible antimitotic agent

complete mitosis and reenter G_1 phase; and wherein the incubation of step (c) is for the time interval determined in step (ii). Another embodiment of the method utilizes non-suspension culture cells from, for example, adherent human or murine cancer cells, harvested by any suitable means for detaching them from tissue culture flasks.

One aspect of the method further includes repeating steps (a) – (f) using a range of relative concentrations of test compound to determine what two substantially minimum concentrations of the test compound provide substantially complete mitotic block in step (d) and in step (f), respectively. The ratio of these minimum sufficient concentrations is an index of reversibility (see detailed U937 protocol for preparation of exemplary dose-response curves). These concentrations may be determined by extrapolating curves of the percentage of cells (from steps (d) and (f)) as a function of concentration (e.g., by testing only a few concentrations, such as 3 or fewer), or by empirically testing a full range of concentrations.

The above methods are useful for identifying an agent (test compound) that inhibits mitosis, for identifying a mitotic blocking agent which substantially retained its mitosis blocking effectiveness after its removal, and for predicting, for example, the IC_{50} or the IC_{95} of a mitotic blocking agent. When compared with relatively reversible antimitotic agents, substantially irreversible antimitotic agents, in other words, agents which continue to block mitosis in a cell which has been only transiently exposed to the agent, are likely to be more effective *in vivo* where natural processes, including multi-drug resistance (MDR) pumps and metabolic or other degradative pathways, prevent prolonged exposure. The effectiveness of relatively reversible antimitotic agents may depend upon a period of sustained exposure.

In view of the cost of developing pharmaceuticals, the economic advantages of determining reversibility ratios, as described above, are considerable. The above methods can be used, for example, to predict whether a test compound with good *in vitro* activity will be effective *in vivo*, such as in a clinical trial. Relatively reversible agents would not be expected to perform as well as irreversible agents. This is shown, for example, by contrasting the data for two known compounds, the relatively irreversible antimitotic agent vincristine and the highly reversible antimitotic agent vinblastine.

Table 2

Reversibility Characteristics of Vinblastine and Vincristine

	Drug concentration required for complete mitotic block, nM			
Compound	0 hour (before washout)	10 hour (after washout)	Reversibility ratio	Interpretation
Vinblastine	10	600	60	Highly Reversible
Vincristine	10	10	1	Irreversible

Analyses of the antimitotic drugs vinblastine and vincristine in the U937 Mitotic Block Reversibility Assay indicate that despite identical potencies to induce initial mitotic blocks (0 hour values), the abilities of the two drugs to induce mitotic blocks which are sustained 10 hour after drug washout (10 hour values) are very different: vincristine induces irreversible mitotic blocks, while those induced by vinblastine are highly reversible.

Analyses of *in vivo* anticancer activities of the antimitotic drugs vinblastine and vincristine against COLO 205 human colon cancer xenografts grown sub-cutaneously in immunocompromised (nude) mice indicate that at equivalent doses of 1 mg/kg, vincristine shows substantial cancer growth inhibitory activity while vinblastine is inactive (FIG. 5). At the lower dose of 0.3 mg/kg, vincristine still produces moderate growth inhibition, while vinblastine is again inactive. The greater *in vivo* activity of vincristine correlates with its irreversibility relative to vinblastine's high reversibility.

E. Use

The disclosed compounds have pharmacological activity, including anti-tumor and anti-mitotic activity as demonstrated in section D above. Examples of tumors include melanoma, fibrosarcoma, monocytic leukemia, colon carcinoma, ovarian carcinoma, breast carcinoma, osteosarcoma, prostate carcinoma, lung carcinoma and ras-transformed fibroblasts.

The invention features pharmaceutical compositions which include a compound of formula (I) and a pharmaceutically-acceptable carrier. Compositions can also include a combination of disclosed compounds, or a combination of one or more disclosed compounds and other pharmaceutically-active agents, such as an anti-tumor agent, an immune-stimulating agent, an interferon, a cytokine, an anti-MDR agent or an anti-angiogenesis agent. Compositions can be formulated for oral, topical, parenteral, intravenous, or intramuscular

administration, or administration by injection or inhalation. Formulations can also be prepared for controlled-release, including transdermal patches.

A method for inhibiting tumor growth in a patient includes the step of administering to the patient an effective, anti-tumor amount of a disclosed compound or composition. The invention also contemplates combination therapies, including methods of co-administering a compound of formula (I) before, during, or after administering another pharmaceutically active agent. The methods of administration may be the same or different. Inhibition of tumor growth includes a growth of the cell or tissue exposed to the test compound that is at least 20% less, and preferably 30%, 50%, or 75% less than the growth of the control (absence of known inhibitor or test compound).

Other Embodiments

The essential features of the invention can be easily discerned from the above description and the claims below. Based on the entire disclosure, variations of the disclosed compounds and methods of the invention described can be designed and adapted without departing from the spirit and scope of the claims and the disclosure. References and publications described herein are hereby incorporated in their entirety.

What is claimed is: